THE OXYTALAN FIBRE IN THE PERIODONTIUM

A THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

by

MILTON REGINALD SIMS, B.D.S., M.Sc.D., F.R.A.C.D.S.

Department of Dental Health
The University of Adelaide
South Australia

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SUMMARY

Knowledge of the organization, metabolic properties, and function of the oxytalan fibre is not extensive. The aims of the present investigation were fourfold. Firstly, to examine histologically the arrangement and distribution of oxytalan fibres in the molar periodontal ligament of the mouse mandible. Secondly, to assess the effects of experimental lathyrism upon the oxytalan fibre. Thirdly, to statistically evaluate the effects of molar intrusion and extrusion on the oxytalan fibre arrangement. And, finally, to compare the oxytalan fibre system in the mouse with that in the maxillary bicuspid region of man.

Findings in the mouse revealed that the oxytalan fibre system associated the vascular system with the teeth, the gingivae, and the insertion of masticatory muscles. The fibres formed a dense three-dimensional meshwork extending from the dentinocemental junction to the peripheral vessels of the periodontal ligament. Distinct regional patterns were observed in the orientation and distribution of the meshwork. A modification of the meshwork in the mid root region suggested adaptation to functional movement of the tooth about a fulcrum. The oxytalan fibre system extended uninterruptedly from the first to the third molar and exhibited a morphology and distribution distinct from that of the principal collagen fibre bundles.

Experimental lathyrism in young albino mice demonstrated marked pathological changes in the periodontal ligament of all molars after seven days. When lathyrism was prolonged for 12 weeks both the molar and incisor oxytalan systems were still readily identifiable although other components of the molar periodontal ligament remained

severely affected by lathyrism. Oxytalan fibres retained their characteristic tooth-vascular association in all lathyritic mice. Findings indicated that in the lathyritic mouse the oxytalan fibre system of functioning teeth possessed a high degree of permanence and was metabolically distinct from collagen and elastic fibres.

A technique was developed to produce experimental tooth movement in the mouse. Intrusion and extrusion of the mandibular first molar in normal and lathyritic mice demonstrated statistically significant changes in the angle of oxytalan fibre attachment to the cementum surface. The data lent support to the hypothesis that oxytalan fibres are capable of independent function within the periodontal ligament.

The periodontal ligament of human premolars and mouse molars revealed similar three-dimensional oxytalan meshworks. Oxytalan fibres displayed two comparable types of vascular association in man and mouse. One arrangement was a generalized relationship with individual periodontal vessels of all types. The second type of association involved the formation of oxytalan-vascular structures in which the oxytalan meshwork enclosed groups of vessels, as well as the individual vessels within each group, to form complex units.

On the evidence produced it is postulated that in the mouse the oxytalan fibre meshwork forms part of a stretch-sensitive proprioceptor mechanism which registers functional tooth oscillation and gingival distortion as tensional variations in the walls of vessels in the periodontal ligament. Since the arrangement and distribution of the oxytalan meshwork is analogous in mouse and man, it is considered that the system serves a similar physiological function in both species.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief, it contains no material previously published or written by any other person except when due reference is made in the text.

MILTON R. SIMS

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CHAPTER I

REVIEW OF LITERATURE

INTRODUCTION

Throughout this review the terminology used by other investigators has been retained. However, in describing his own studies the author has adopted the term periodontal ligament as advocated by Noble (1969). Furthermore, the author concurs with the view of Melcher and Eastoe (1969) who state that while separation of the soft tissues of the periodontium into periodontal ligament and gingival lamina propria may have descriptive advantages, the two tissues should be regarded as two anatomical parts of the same functional system. Because of this functional connotation the author refers to both of these anatomical parts of the soft connective tissues as the periodontal ligament.

The oxytalan fibre in the periodontal ligament

During an investigation of age changes in connective tissues, Fullmer (1958) reported the selective staining of a new type of fibre in the human periodontal ligament which had formerly been considered to be composed almost entirely of collagen. Following peracetic acid oxidation of formalin fixed tissue sections, these new fibres stained a brilliant purple colour with aldehyde fuchsin, but lacked the birefringent properties of the unstained collagen fibres.

Fullmer described the anatomical arrangement of the vividly stained fibres stating that they were anchored either in the cementum or bone at one end and frequently branched as they followed the course

of the principal collagen fibres. Single fibres extending from tooth to bone could not be identified. In the gingival and transseptal regions the fibres were orientated in the same direction as the collagen fibres. However, throughout the gingival tissues and the periodontal ligament many of these purple staining fibres exhibited a multidirectional distribution.

The fibre was demonstrated in human tendons, ligaments, the adventitia of blood vessels, the connective tissue sheath surrounding hair follicles, and the epineurium and perineurium. These fibres were also observed in the periodontal ligament of mice, rats and guinea pigs, as well as in the Achilles tendons and/or patellar ligaments of monkeys, mice, rats, guinea pigs and the turkey. Fullmer further distinguished the new fibres from elastic and reticular fibres by their different staining properties and the susceptibility of elastic fibres to elastase digestion.

Fullmer and Lillie (1958) named these new connective tissue structures "oxytalan" fibres because of their resistance to acid hydrolysis. Oxytalan fibres were examined in human, rat, mouse, monkey and guinea pig tissues. Details of the peracetic acid-aldehyde fuchsin staining method were given. A comparison was made of the effects of various staining reactions and enzyme hydrolysis with elastase, hyaluronidase, and lysozyme, upon the oxytalan and elastic fibre. The results of acid and alkali solubility tests and different blocking reactions were also reported. It was reaffirmed that the distribution of oxytalan fibres appeared to be restricted to sites where connective tissues were subjected to stress, and that oxytalan fibres were distinct from the collagenous, elastic, and reticular varieties. The authors observed that the oxytalan fibre did not react when the regular elastic stains were applied, nor did staining for collagen or reticulin

selectively demonstrate oxytalan fibres. However, when sections were oxidized with peracetic acid, three of the five elastic tissue stains - Taenzer-Unna orcein, Gomori's aldehyde fuchsin, and Weigert's resorcin fuchsin - would react with oxytalan fibres whereas the Verhoeff and orcinol-new fuchsin stains did not.

Fullmer and Lillie suggested that the oxytalan fibre contained a reduced form of some component of the elastic fibre which did not react with elastic staining methods, and that peracetic acid oxidation was required to produce the reactive grouping(s). Despite a variety of investigations the exact nature of the reactive groups responsible for the peracetic acid-aldehyde fuchsin staining process remained unanswered.

The authors proposed that mucopolysaccharide components were responsible for the staining of both elastic and oxytalan fibres. In the case of the oxytalan fibre, it was thought that peracetic acid might act to break down the mucopolysaccharide-protein link and thereby liberate groups susceptible to certain methods of staining and enzyme digestion by lysozyme and testicular hyaluronidase. Fullmer and Lillie stated in their conclusions that after peracetic acid oxidation the stainable material in oxytalan fibres was slowly removed by lysozyme and by testicular hyaluronidase. This reaction was not prevented if the digestion period preceded the oxidative step. Elastic fibres similarly treated showed no observable effect. Formalin fixed oxytalan fibres were digested by elastase only if they were pre-oxidized with peracetic acid.

In a subsequent publication, Fullmer (1959a) detailed the use of the peracetic-orcein-Halmi stain as a selective stain for the demonstration of oxytalan and elastic fibres. The method was stated to be more specific than the peracetic-aldehyde fuchsin technique in

the staining of mucopolysaccharides. Different fixatives were used for dental tissues obtained from human embryos and individuals up to the age of 79 years. Pulp fibrils reactive with the peracetic-orcein method were not demonstrated by the use of stains for collagen, elastin, reticulin or acid mucopolysaccharide. It was stated that developing elastic fibres in the human embryo could be visualized with the peracetic-orcein and peracetic-aldehyde fuchsin stains prior to their demonstration by any of the regular elastic tissue stains. Fullmer considered that the elastin-precursor substance reactive with the peracetic-orcein stain might be a reduced form of mucoprotein-like substance which, concurrent with maturation and the incorporation of other substances, became elastin.

The development of oxytalan fibres in the periodontal ligament of man was studied by Fullmer (1959b). He employed a variety of staining methods for acid mucopolysaccharides, collagen, elastic and oxytalan fibres, and protein. The effects of the oxidants peracetic acid and periodic acid were also observed. Jaw sections of embryos demonstrated acid mucopolysaccharides and collagen prior to oxytalan fibres. When oxytalan fibres were first observed stains for acid mucopolysaccharides reacted strongly between collagen bundles. Oxidation with peracetic acid prior to the Rinehart stain demonstrated additional reactive material. Peracetic acid also increased the amount of orthochromatic material observed with azure A. Initial formation of oxytalan fibres was demonstrated only with the peracetic acid-aldehyde fuchsin-Halmi or the peracetic acid-orcein-Halmi stains whereas the mucopolysaccharide surrounding the fibres was revealed with the azure A, Rinehart, peracetic acid-Rinehart and peracetic acid-aldehyde fuchsin stains.

Oxytalan fibres were observed to develop in two locations.

The fibres were first detected in the oral mucosa of a six month embryo occlusal to the developing tooth where they became fibres of the gingivae. The second group of oxytalan fibres arose adjacent and peripheral to the outer enamel epithelium to become fibres of the periodontal membrane or gingivae. It was assumed that the second fibre group arose after the six month embryo stage as they were found to be absent at this stage but present surrounding the outer enamel epithelium of the four month old child.

During cementogenesis oxytalan fibres were embedded in the cementum from the cementoenamel junction to the apex. As the vascular system became established in the periodontium oxytalan fibres were said to extend from the vessel adventitia to the cementum. Fullmer drew attention to changes in the staining properties of the mucopolysaccharides of the ground substance at different ages during development. He suggested that the mucopolysaccharides might combine with protein or other substances to contribute to the formation of oxytalan, elastic and collagen fibres, thereby making the reactive groups required for the azure A and peracetic-Rinehart stains no longer available.

Fullmer (1960b) described the effects of peracetic acid upon the enzymatic digestion of various mucopolysaccharides including the reversal of the PAS staining reaction for mucin. Animal and human tissues were exposed to selected stains after enzyme digestion with and without prior oxidation with peracetic acid. Peracetic acid oxidation enhanced the staining of all structures that reacted with aldehyde fuchsin without the oxidative step and induced the staining of oxytalan fibres. In sections preoxidized with peracetic acid the enzymes β-glucuronidase, hyaluronidase, lysozyme, elastase and trypsin digested periodontal oxytalan fibres and prevented their staining with aldehyde fuchsin. Elastic fibres stained strongly with the direct application of

aldehyde fuchsin and also when this dye complex was applied after the various oxidation-enzyme and enzyme-oxidation tests.

Fullmer referred to the histochemical significance of the peracetic acid-aldehyde fuchsin-Halmi staining reaction, and reviewed variations found in the degree of staining of different mucopolysaccharides. The digestive properties of the different enzymes were compared but the oxidizing mechanism of peracetic acid remained undetermined. The peracetic acid-aldehyde fuchsin-Halmi stain, in conjunction with β-glucuronidase digestion, was recommended for the study of connective tissues.

A comparative histochemical study of elastic, pre-elastic and oxytalan connective tissue fibres in the periodontal ligament of several mammals was published by Fullmer (1960c). Oxytalan fibres were said to be related to elastic fibres on the basis that (a) they stained with three of five elastic tissue stains if preoxidized with peracetic acid, (b) they were digested more readily than collagen when commercial elastase was applied after peracetic acid, (c) elastic fibres were a regular constituent of the periodontium of some animals whereas oxytalan fibres were present without elastic fibres in other animals, (d) oxytalan fibres were located in specially modified connective tissue structures devoid of elastic fibres wherein one could logically assume that a modified elastic product might be produced. Connective tissue fibres that stained with elastic tissue stains and were digested by elastase were identified as elastic fibres.

Fibres were designated oxytalan fibres if they fulfilled all of the following criteria: (a) failed to stain with elastic tissue stains, (b) stained with either the peracetic acid-aldehyde fuchsin-Halmi or the peracetic acid-orcein-Halmi methods, (c) failed to stain

by the staining reactions listed under (b) when a β-glucuronidase or a testicular hyaluronidase digestion was interposed between the oxidative and the staining steps, and (d) resisted elastase digestion.

The proportion of oxytalan to elastic fibres was shown to vary according to the species, the type of tooth and its location. However, in the artiodactyla a clear distinction could not be made as portions of a single fibre reacted differently with some stains and enzyme digestions. Criteria were detailed for distinguishing between elastic, pre-elastic and oxytalan fibres. The view was expressed that oxytalan fibres might represent an immature or a specially modified elastic fibre. Pre-elastic fibres could not be differentiated from oxytalan fibres on the basis of the histochemical methods used. It was proposed that the term oxytalan fibre should designate elastic-like fibres in certain structures that in the adult never developed into characteristic elastic fibres. The term pre-elastic fibre was assigned to either developing elastic tissue in structures where it was expected to undergo maturation into characteristic elastic fibres, or to extensions of recognizable elastic fibres.

In a study of periodontal disease in the human maxilla Fullmer (1961) included new data on the distribution of oxytalan fibres in normal periodontal structures. Mesiodistal sections through the transseptal region showed that oxytalan fibres were inserted in the cementum and gradually decreased in size as they extended outward with the collagen fibres to occasionally arborize before terminating at varying distances from the tooth. Oxytalan fibres were said not to extend to adjacent teeth or adjacent bone thereby leaving an interproximal zone usually devoid of the fibres. In the gingiva many oxytalan fibres extended from the cementum and accompanied the free gingival fibre groups whereas others encircled the tooth in a formation which could extend into the transseptal

regions. Some oxytalan fibres extended into the basement membrane of the epithelial attachment and passed into the subepithelial connective tissues where they arborized before termination. A concentration of oxtyalan fibres was usually seen immediately below the epithelial attachment.

Oxytalan fibres were said to be inserted into bone but not to span the width of the periodontal ligament. Although the fibres coursed in many directions the largest and greatest number of them were found on the tooth side of the ligament. Deciduous periodontal ligaments contained many large oxytalan fibres aligned in an occluso-apical direction. Some fibres extended from lymphatic vessels to the cementum. In the apical region oxytalan fibres were stated to be multidirectional and to frequently form a network. The periodontal ligament was considered to be a distinctive connective tissue structure which differed from skin and connective tissue generally by the absence of elastic and the presence of oxytalan fibres. Furthermore, the periodontal ligament was distinguished by having a large mucopolysaccharide content together with a high degree of organization and orientation of the fibrous components. These characteristics were said to relate the periodontal ligament to tendons and ligaments.

Fullmer (1962) reviewed the normal connective tissue components of the periodontium and alterations associated with periodontal disease. Emphasis was given to the structure, chemical composition, metabolic behaviour and staining reactions of collagen. The response of elastic and oxytalan fibres to selective dyes and enzymatic hydrolysis was outlined, while the functional role of the oxytalan fibre and mucopolysaccharides was discussed. It was noted that some cases of periodontal disease revealed the persistence of oxytalan fibres in an inflammatory lesion after the immediately surrounding collagen had been destroyed. Subsequently, however, the oxytalan fibres were also

destroyed. In the absence of periodontal disease, no alterations to oxytalan fibres were seen in the periodontal ligament of individuals from the middle of the second to the eighth decade in age.

Kohl and Zander (1962) examined oxytalan fibre arrangements in the interproximal tissues of adult Rhesus monkeys. These investigators confirmed the arrangement and distribution observed by Fullmer (1961) in the human, and described a palisade arrangement of the fibres in vestibulo-lingual sections. Where inflammatory lesions were present collagen was denatured before the oxytalan fibres. Surgical removal of the interdental gingivae was said to result in regeneration of the oxytalan fibres and other connective tissue components after two months.

Rannie (1963) investigated oxytalan fibres in the human, monkey and rat periodontal ligament and agreed with Fullmer that the fibres penetrated the alveolar bone. Rannie introduced potassium monopersulphate as an oxidant to replace peracetic acid. He reported consistent staining of oxytalan fibres with methylene blue, toluidine blue and celestin blue. The specificity of elastic tissue stains was questioned by him and the relationship of oxytalan fibres to elastic structures considered. Oxytalan fibres were claimed to be embedded in bone and cementum.

Reference was made by Fullmer (1963) to histochemical evidence which indicated that the oxytalan fibre probably represented a type of elastic tissue. The oxytalan fibre was believed to contain a protein and mucopolysaccharide component, the latter being enzyme digestable provided the fibres were preoxidized with peracetic acid. In the adult the periodontal connective tissue demonstrated the capacity to produce oxytalan fibres in pathological conditions. Oxytalan fibres appeared to be more resistant than collagen to periodontal disease.

Löe and Nuki (1964) examined oxytalan fibres in the periodontal structures of a dog, adult Rhesus monkeys and humans. The materials and methods section was preceded with an inaccurate summary of the findings of Fullmer and his co-workers. Löe and Nuki found that oxytalan fibres stained haphazardly with the aldehyde fuchsin routines they used and, therefore, they erroneously reported a scarcity of the fibres in the attachment apparatus. These investigators concluded that oxytalan fibres were neural elements since the aldehyde fuchsin also stained motor end plates and because they found a similar morphology and distribution of both oxytalan-positive and argyrophilic fibres which in their opinion corresponded closely with that of the neural elements described in the periodontal ligament by Bernick (1957).

Fullmer (1964) stressed that collagen, mucopolysaccharides, bones and developing teeth were present in the jaw before oxytalan fibres were seen. This particular description of oxytalan fibre development was based on an earlier (1959b) paper.

In a chapter describing the use of histochemistry in oral pathology Fullmer (1965a) compared the elastic and oxytalan fibre. He reiterated that the oxytalan fibre was a normal constituent of the human periodontal ligament and distinct from elastic, reticular and collagenous fibres. The data which revealed the relationship of oxytalan fibres to elastic fibres was listed. It was stated that since various staining reactions failed to differentiate pre-elastic from oxytalan fibres this provided evidence to supplement the hypothesis that oxytalan fibres represented immature or specially modified elastic-like fibres. Data was said to indicate that peracetic acid-aldehyde fuchsin-Halmi stained mucopolysaccharide in developing teeth which was not revealed by other methods. Reference was made to the presence of oxytalan fibres in various pathological conditions and it was presumed that scleroderma

resulted in the overmaturation of the elastic system of fibres such as oxytalan. This observation was said to support a relationship between oxytalan and elastic fibres. Fullmer stated that any study of the connective tissues of the jaws was incomplete without the inclusion of a study of oxytalan fibres and the realization of the existence of a spectrum of elastic and elastic-like tissue.

In considering the histochemistry of connective tissues Fullmer (1965b) extensively reviewed elastic fibres with respect to their morphology, distribution, biochemical composition, staining properties, mechanisms of staining, and the reactive groupings involved. He stated that a definitive relationship of elastic to oxytalan and pre-elastic fibres had not been established. While the staining reactions of pre-elastic and oxytalan fibres were said to be analogous, the fibres had not been analyzed and shown to have the same chemical composition. Fullmer justified the use of the terms oxytalan fibres and pre-elastic fibres although he mentioned that staining methods did not permit a clear distinction between pre-elastic and oxytalan fibres. He expressed the opinion that the term pre-elastic should be restricted to either those fibres which could be expected to mature eventually into elastic fibres or those that were extensions of recognizable elastic fibres.

Performic acid, bromine, and acid permanganate were mentioned as substitute preoxidants for peracetic acid and Oxone in oxytalan fibre identification. The function of peracetic acid in the induction of oxytalan fibre staining with aldehyde fuchsin and the digestibility of the fibre with commercial elastase and β-glucuronidase was said to be unknown. Fullmer referred to several of his earlier studies, particularly those relating to enzyme hydrolysis. The presence of oxytalan fibres in certain pathological conditions brought forward the suggestion that these fibres represented elastic-like fibres produced by connective tissue cells

in lieu of typical elastic fibres.

A description of oxytalan fibres in the deciduous dentition was published by Goggins (1966) who considered their distribution to be essentially similar to that found in the permanent dentition. However, differences reported included a greater number of oxytalan fibres in the cementum of permanent teeth and the presence of a network of fine oxytalan fibres adjacent, but unattached, to the deciduous cementum. Goggins disputed Löe and Nuki's neural concept for oxytalan fibres on the grounds of inadequate staining techniques, inaccurate correlation of results, assumed specificity of aldehyde-fuchsin staining, and misinterpretation of the findings reported by Fullmer and Lillie (1958), Fullmer (1959b, 1960a, 1960c, 1961, 1962), and Bernick (1957, 1959).

Fullmer (1966a) reviewed the oxytalan fibre, its staining characteristics, identification from other connective tissue fibres, response to enzyme digestion, and the results of electron microscope studies. Findings of previous investigations of oxytalan fibre morphology, distribution, and insertion into bone were reaffirmed. The relationship of oxytalan fibres to elastic fibres in man and animals led to the conclusion that oxytalan fibres were possibly pre-elastic or specially modified elastic fibres.

The structure of normal and disease affected connective tissues was reviewed by Fullmer (1966b). He remarked that while elastic fibres occurred in normal connective tissues, including the gingivae and oral mucosa, these fibres were found only in association with vascular structures in normal periodontal membranes. Outside of the vascular structures modified elastic-like (oxytalan) fibres were formed in the periodontal ligament. In abnormal connective tissues such as periapical abscesses, dental granulomas and radicular cysts, oxytalan fibres only

developed in the more mature regions characterized by the presence of abundant coarse collagenous fibres. Where scleroderma affected the periodontium, a proportional increase of oxytalan, collagen and elastic fibres developed in regions normally containing only oxytalan fibres. However, elastic fibres did not develop in uninvolved periodontal ligaments of the same individual. This observation was said to support the concept of the relation of elastic to oxytalan fibres.

Fullmer (1967a) briefly outlined his earlier studies on oxytalan fibre development. In another publication describing connective tissue components of the periodontium Fullmer (1967b) comprehensively reviewed oxytalan fibre formation, morphology and distribution. He considered the staining properties of the fibres, their reaction to enzyme hydrolysis, and presented a comparative study of oxytalan, reticular and elastic fibres in animals and man. Reference was made to scanning electron microscope studies of oxytalan fibres and the hypothesis was put forward that oxytalan fibres represented an arrested development of elastic fibres.

Beynon (1967) studied the development and distribution of oxytalan fibres in the mouse molar during early root formation. Oxytalan fibres were demonstrated inserted into the cementum at the level of break-up of Hertwig's root sheath, lining the pulpal surface of the epithelial diaphragm, and passing between the odontoblasts in the coronal pulp to become obscured in predentine. It was indicated that the developing oxytalan fibrils were analogous with the nonstriated fibrils reported by Selvig (1963) and Carmichael and Fullmer (1966).

Simpson (1967) examined oxytalan and elastic fibres in fragments of periodontal ligament obtained from extracted human teeth. Oxytalan fibre morphology, arrangement and density variation were described.

Simpson called the main oxytalan fibres anchoring fibres and described three other fibre types. These included a group of fibres of variable size interlacing among the other structures of the ligament. A second fibre type accompanied the collagen bundles as if reinforcing them. Thirdly, there was a fine network of elementary fibrils best observed near the cemental surface and often difficult to see because of its tenuous nature.

Soule (1967) demonstrated oxytalan fibres in the periodontal ligament of the Caiman and the Alligator thus indicating that the fibres were not restricted to mammals. Oxytalan fibres extended a short distance into the periodontal ligament from the cementum but no oxytalan fibres were observed extending into the periodontal ligament from the alveolar bone.

Carmichael (1968) examined the distribution and connections of the oxytalan fibre in the lower jaw of the mouse. His staining methods, which were said to outline cell membranes and processes, led him to suggest that the oxytalan fibres were intimately related to these structures and the periodontal vessels. Oxytalan fibres were generally orientated obliquely across the periodontal ligament and frequently arranged perpendicular to the collagen fibre bundles. The oxytalan fibre arrangement was said to be oblique to the axial and lateral compressional forces exerted by the tooth on the periodontal ligament.

The effect of orthodontic rotation upon the oxytalan fibre system of dogs' teeth was investigated by Edwards (1968). He described the oxytalan fibre arrangement associated with the free gingival collagen fibre group but stated that none of the oxytalan fibres in the transseptal region could be traced from one tooth to the next. Furthermore, the centre half of the septal area was reported to be

completely devoid of oxytalan fibres in both the control and experimental tissues. Oxytalan fibres were not observed extending into the alveolar crest although it was stated that beneath the transseptal region practically none of the oxytalan fibres extended in a horizontal direction from tooth to bone. Edwards considered it significant that oxytalan fibres, particularly in the supracrestal areas, were larger and more numerous in the tissues of rotated teeth than control teeth. Retention for five months was said not to eliminate this preponderance of oxytalan fibres in the gingival tissues of the rotated teeth. He believed that the increase in oxytalan fibres was associated with a build up in elastic force.

Soule (1969) showed that the periodontal ligament of balistids contained a few, delicate oxytalan fibres. These fibres were most abundant in the coronal region beneath the interdental papillae and thus resembled the distribution in mammals. No mention was made of oxytalan fibre insertion into the cementum.

Boese (1969) rotated teeth in two adult monkeys and found the greatest concentration of oxytalan fibres in the transseptal and alveolar crest areas of all experimental and control teeth. Oxytalan fibres were reported to form a continuous network extending transseptally between rotated and adjacent teeth, but not to span the transseptal area of normal teeth. The attachment of branched oxytalan fibres to alveolar bone was described in both experimental and control tissues. Tooth rotation was said to be accompanied by a marked increase in the number of oxytalan fibres in the transseptal area, particularly of rotated controls which had been allowed to relapse. It was reported that oxytalan fibres in specimens prepared after 4 weeks were straighter and appeared to be under more tension than teeth which had relapsed for 8 weeks.

Miake, Higashi, Machida, Ito and Ogawa (1970) described the arrangement of elastic fibres in the periodontal ligament and cementum of human deciduous teeth. In their introduction the authors stated that the fibrous elements of the connective tissue were in general reticular, collagenous and elastic fibres. No mention was made of oxytalan fibres or preoxidation of the sections prior to staining with elastic tissue stains. However, the reported elastic fibre morphology and distribution was strikingly similar to Goggins' (1966) description of oxytalan fibres in the deciduous dentition.

Edwards (1971) examined the gingival tissue of teeth orthodontically approximated across extraction spaces. He reported an enormous increase in the number of oxytalan fibres in the unattached gingival papillae of the displaced tissues. The article stated that the increase in gingival oxytalan density produced during tooth rotation did not disappear after one year of retention. However, the investigation quoted in support of this claim (Edwards 1968) did not continue retention beyond eight months.

Oxytalan fibres in the armadillo were described by Shackleford (1971) as generally being orientated parallel to the long axis of the teeth and inserted into the cementum. The armadillo oxytalan fibre pattern was said to be similar to that of human deciduous teeth and the incisor teeth of rats and guinea pigs.

Roche (1972) observed oxytalan fibres in the gingival col of children. The fibres showed an irregular pattern and exhibited great variation in density and distribution from specimen to specimen. Oxytalan fibres were often orientated in the same direction as collagen fibre bundles but sometimes aligned at right angles to them.

Vital maxillary incisors of monkeys were endodontically treated

and reimplanted by Hurst (1972). One week after reimplantation oxytalan fibres were prominent throughout the periodontal ligament including the transseptal region. After two weeks oxytalan fibres in both regions were said to have completely regenerated. The onset of ankylosis after three weeks was associated with a progressive and marked decrease in the size and number of oxytalan fibres. Elastic fibres were only observed in the submucosa.

Parker (1972) bodily retracted teeth in the Macaca rhesus. The oxytalan fibre was found to be most numerous in areas of stress such as the transseptal region. Oxytalan fibres attached to the cementum just below the cementoenamel junction followed the configuration of the collagen fibres becoming less numerous as they approached the alveolar crest. The oxytalan fibres were not seen to be attached to bone, crossing the alveolar crest, or connecting approximating teeth. Elastic fibres were said to be absent in the rhesus periodontal ligament.

A significant paper was published by Sheetz, Fullmer and Narkates (1973) who identified with light and electron microscopy the same oxytalan fibre in serial sections of the rat periodontal membrane.

Knowledge of the oxytalan fibre was summarized by Fullmer, Sheetz and Narkates (1974) who reaffirmed that it was a separate and distinct fibre type indistinguishable from pre-elastic fibres with current histochemical methods but readily distinguished by electron microscopy. The relationship of the oxytalan fibre to the elastic fibre was considered from the aspects of its staining properties, its presence in the periodontal tissues of a patient with scleroderma, enzyme digestion experiments, and its staining characteristics in developing dental tissues. The ultrastructural properties of oxytalan and elastic fibres were documented, reference was made to the selective staining properties

of aldehyde fuchsin, and a recommended staining method was given for the use of this dye with light microscopy.

Features of the oxytalan fibre were compared with those of collagen, reticulum, elastic and pre-elastic fibres. Oxytalan fibre distribution, function, composition, histogenesis, presence in dental reparative and neoplastic tissues, as well as the presence of the fibre in non-dental sites, were outlined. The authors emphasized that oxytalan fibres increased in number and size in the periodontal ligament of teeth subjected to increased stress from bridge abutments and orthodontic forces.

A study by Oxberry (1975) of the periodontal ligament of the continuously erupting molars of the vole revealed elongated granular bodies about 2 μm in length which were oxytalan fibre stain-positive. The molar cementoid buttresses were stated to contain a few oxytalan and elastic staining fibres. Oxytalan positive elements were also seen in the reparative dentine. It was suggested that the elongated granules might be a rudimentary stage of oxytalan fibres.

Sampson (1975) examined the oxytalan fibre arrangement in a group of Australian marsupials comprising a wallaby, wombat, possum and marsupial mice. The oxytalan fibre proved to be a normal component of all periodontal ligaments forming a system which invested each tooth and linked adjacent teeth transseptally.

An abundant network of fine branching oxytalan fibres extended between the cementum and vessels in all the marsupials. Above the level of the alveolar crest the oxytalan fibres were predominantly orientated with the principal collagen fibre system. Within the periodontal ligament fibres cascaded apically and oblique to the principal collagen fibres. There were also long ribbon-like

fibres orientated perpendicular to the collagen fibre bundles and extending from the cervical to the apical third region.

Soames and Davies (1975) examined normal and inflamed dog gingivae for elastic and oxytalan fibres. Oxytalan staining fibres were found in the basement membrane regions of oral and junctional epithelium and around the small vessels. Where collagen fibres were destroyed by inflammation the oxytalan fibres persisted.

The occurrence of oxytalan fibres in the periodontal ligament of partially erupted teeth, erupted teeth and orthodontically intruded teeth was studied in mini-pigs by Markens and Oudhof (1976). Oxytalan fibre length, their numbers and orientation, were said to differ at various stages of eruption.

The oxytalan fibre and pathological states

An abundance of oxytalan fibres was described at the dermal-epidermal junction by Hasegawa (1960) in biopsy specimens of various skin diseases. Oxytalan fibres were concluded to be capable of regeneration following injury.

Fullmer (1960a) reported that oxytalan fibres developed in dental granulomas and radicular cysts in a manner similar to that previously observed during embryogenesis. The findings were said to indicate the periodontal origin of the connective tissue cells that produced the oxytalan fibres.

The presence of oxytalan fibres in sclerosing haemangiomas was described by Tedeschi and Sommers (1961) as being characteristic of this lesion. However, the relations of the oxytalan fibres to the blood vessels were given scant attention. Fullmer (1961, 1962) suggested that collagen fibres underwent degradation before oxytalan

fibres during the initial stage of periodontal disease.

Tedeschi and Sommers (1962) reported the presence of oxytalan fibres in dermal fibromas and giant-cell tendon sheath tumours. These authors disputed Hasegawa's (1960) findings stating that pressure and stretch phenomena apparently stimulated the oxytalan fibre formation he observed.

An interesting study by Fullmer and Witte (1962) detailed changes observed in the periodontal membrane affected by scleroderma. Contrary to normal, elastic fibres having a collagen fibre orientation were prevalent adjacent to the teeth in regions of collagen sclerosis and hyalinization.

Oxytalan fibres were also reported to be present in ameloblastomas by Fisher and Fullmer (1962). A feature was the finding that elastic fibres in the lesions were found only in association with vascular structures.

Hamner and Fullmer (1966) verified the presence of oxytalan fibres in benign fibro-osseous jaw lesions. Kanouse (1966) declared that oxytalan fibres were unaffected by ascorbic acid deficiency in guinea pigs and Baratieri (1967a, 1967b) confirmed the presence of the fibre in the gingival tissues of patients treated with sodium diphenyl-hydantoin. In 1974 Baratieri and Miani described the distribution of oxytalan fibres in tuberous sclerosis lesions of the oral mucosa.

The presence of oxytalan fibres has moreover been reported in the periodontal ligament of lathyritic animals by Gardner (1960) and Kennedy and Kennedy (1963).

These studies establish that the oxytalan fibre is a

component of both the normal and abnormal periodontal ligament.

The oxytalan fibre in non-dental sites

Gawlik and Jarocińska (1964) described oxytalan fibres in human tendons and Gawlik (1965) based his classification of elastic elements as oxytalan, elaunin, elastic, or pre-elastic fibres, on the staining of human connective tissues.

Carmichael and Oakes (1970) reported the presence of elastic fibres and fibres resembling oxytalan fibres in the periarticular tissues of the rat. Both elastic and oxytalan fibres were demonstrated in the rabbit temporomandibular joint by Duthy (1972). In 1975 Luke published his observations of oxytalan-type fibres in the developing human and equine temporomandibular joint.

Cameron, Jennings and Rannie (1970) and Dawes (1970) described oxytalan fibres in the human tympanic membrane. Cotta-Pereira, Rodrigo, and David-Ferreira (1976) claimed to be able to distinguish oxytalan, elaunin and elastic fibres in human skin.

Thus the original finding by Fullmer (1958) that oxytalan fibres occur in a variety of connective tissues subject to stress has been confirmed and amplified.

Electron microscope studies

Fullmer (1966a) referred to electron microscope studies of the oxytalan fibre made by Selvig (1963) and by Goggins (1965) in the periodontal ligament of man. The appearance of the fibres in man and rat was considered indistinguishable being composed of many fine filaments with an amorphous interfibrillar material of similar diameter. Periodicity was not detected. The electron microscope confirmed that the oxytalan fibre differed from other connective tissue fibre types.

The ultrastructure of the oxytalan fibre in the periodontal ligament of the rat incisor was detailed and illustrated by Carmichael and Fullmer (1966). Oxytalan fibres were said to consist of bundles of filaments approximately 15.0 nm in diameter gathered together with an interfilamentous amorphous substance of a corresponding diameter. The filaments did not show regular periodicity and were of indefinite length. A morphological similarity was noted between the ultrastructure of the oxytalan and elastic fibre although a size discrepancy existed.

Greenlee, Ross and Hartman (1966) examined the fine structure of developing elastic fibres. The publication included an illustration depicting an immature elastic fibre in the rat flexor tendon which, as the authors admitted, showed a striking resemblance to a photomicrograph of the oxytalan fibre published by Carmichael and Fullmer (1966).

Oxytalan fibres were occasionally observed by Griffin and Harris (1967) in the developing human periodontium obtained from surgically removed third molars. These fibres were said to differ from those described by Carmichael and Fullmer (1966) in consisting of irregularly beaded microfibrils with diameters which varied between 7.5 nm and 17.5 nm, and the presence of an amorphous component surrounding the beaded elements. It was suggested that the oxytalan fibril could consist of a protein-polysaccharide complex and form, as a result of end to end linkage and lateral aggregation of the ground substance, microfibrils.

A paper by Harris and Griffin (1967) reported that periodontal microfibrils and oxytalan fibrils of the human periodontium were partially denatured by peracetic acid oxidation. Subsequent treatment with β-glucuronidase disrupted both microfibrils and oxytalan fibrils suggesting they were similar in nature. The results indicated that the

microfibrils and oxytalan fibrils were protein-polysaccharide complexes in which the amorphous component corresponded to the polysaccharide moiety.

Oxytalan fibres were recorded in the developing human tooth pulp by Provenza, Fischlschweiger and Sisca (1967). The fibres averaged 15.0 nm in diameter and many exhibited beading.

Selvig (1968) described the presence of nonbanded fibrils in normal and diseased periodontal ligaments. Because of their location and appearance it was considered that the nonbanded structures were partially decomposed collagen fibrils.

Attempts to identify the oxytalan fibre with the scanning electron microscope have proved unsuccessful. Using this instrument Shackleford (1971) was unable to distinguish this fibre in the periodontal ligament of dogs, armadillos and rats.

Sheetz, Fullmer and Narkates (1973) confirmed the characterization of oxytalan fibres at the electron microscope level by identifying the same oxytalan fibre in the rat periodontal ligament with light and electron microscopy. Moreover, the transmission electron microscopic appearance of the oxytalan fibre corresponded with that illustrated by Carmichael and Fullmer in 1966.

Fullmer et al. (1974) reaffirmed that the oxytalan fibre displayed two distinctive features at the ultrastructural level. One was the organized assemblage of fibrils arranged parallel to the long axis of the fibre. The other feature was the variable amount of interfibrillar amorphous material. Fibril diameter was confirmed as being approximately 15.0 nm. A characteristic of the oxytalan fibres examined was said to be the retention of recognizable fibrils in mature fibres. The unique structure of the oxytalan fibre was stated

to delineate it from collagen, reticulin, basement membrane, nerve, elastic and pre-elastic fibres.

The existence of an elaunin fibre (Gawlik 1965) has been ignored in the literature until the recent publication of Cotta-Pereira et al. (1976) who asserted that oxytalan, elaunin, and elastic fibres could be distinguished with the electron microscope.

Oxidation for oxytalan fibre staining

Tissue oxidation is an essential prerequisite for the identification of oxytalan fibres with aldehyde fuchsin, orcein and resorcin fuchsin (Fullmer 1958, 1959a, 1959b; Fullmer and Lillie 1958; Fullmer et al. 1974). Oxidation is also required with other oxytalan fibre stains (Rannie 1963; Gawlik and Jarocińska 1964; Mander, Mander and Carmichael 1968; Sampson 1975).

Greenspan's (1950) peracetic acid used for 10 to 30 min was the first oxidant technique described (Fullmer 1958; Fullmer and Lillie 1958). Fullmer and Lillie (1958) were unsuccessful in their attempts to stain oxytalan fibres with aldehyde fuchsin after pretreatment with mineral and acetic acids and other oxidizing agents including H_2O_2, $FeCl_3$, and periodic acid. Sporadic and inconsistent results were reported with 0.1% aqueous $KMnO_4$ at 25°C for 30 min. Oxidation for 30 min in fresh 3.0% $KMnO_4$ in glacial acetic acid was said to give fairly good results. The following year Fullmer (1959b) reported the successful use of aqueous periodic acid for 10 min at 25°C. Nevertheless, peracetic acid has been used for the majority of oxytalan fibre investigations (Fullmer 1958, 1959a, 1959b, 1960a, 1960b, 1961, 1962, 1963, 1965a, 1965b, 1966b, 1967a, 1967b; Fullmer and Lillie 1958; Fullmer and Witte 1962; Kohl and Zander 1962; Löe and Nuki 1964; Gawlik and Jarocińska 1964; Gawlik 1965; Goggins 1966; Hurst 1972; Cotta-Pereira et al. 1976).

In 1963 Rannie introduced a 10% aqueous solution of Oxone (potassium monopersulphate) as an improved oxidant in lieu of peracetic acid. Performic acid and bromine were also reported to be suitable oxidants (Fullmer 1965b). Thereafter, Fullmer (1966a, 1966b, 1967a, 1967b), Carmichael and Fullmer (1966), and Hamner and Fullmer (1966) mentioned both peracetic acid and Oxone for use with aldehyde fuchsin-Halmi and orcein-Halmi. Fullmer (1965b, 1966a, 1966b) expressed the opinion that Oxone was the preferred oxidant as it did not cause tissue destruction. Subsequently, Fullmer (1967b) reported that strong oxidants such as peracetic acid, performic acid, $KMnO_4$ or Oxone, in conjunction with aldehyde fuchsin, proved to be one of the most important stains for the investigation of the supporting tissues of the teeth.

Mander et al. (1968) declared that both periodic acid and weak potassium permanganate could be used as suitable oxidants for mouse tissues. However, these authors based their investigations largely on the use of tissues oxidized with periodic acid. The claim by Mander et al. that periodic acid was a suitable oxidant was at variance with the opinion of Fullmer and Lillie (1958) and Fullmer (1967b).

A paper by Carmichael (1968) described satisfactory oxidation after 15 min with either a 10% solution of Oxone or a 0.25% solution of potassium permanganate pH 2.0 at room temperature. Shortly afterwards, Carmichael (1969) advocated the use of 10% aqueous potassium monopersulphate for 10 to 15 min at room temperature.

Fullmer (1969) advised oxidation with either peracetic acid or Oxone for 30 min to 1 h at 25^{o}C without recognized loss of stainability with aldehyde fuchsin. He said that peracetic acid was highly unstable after a few hours at room temperature and suggested that

less trouble would be experienced with Oxone. A further recommendation from Fullmer (1972) stated that compared with peracetic acid, Oxone was more convenient, gave the same results and preserved better tissue integrity. Fullmer et al. (1974) reaffirmed their preference for the use of 10% aqueous Oxone for 30 to 60 min at 25^{o}C.

Almost twenty years have passed since the oxytalan fibre was first discovered yet there is a lack of definitive information on the chemistry of the oxidation procedures used for its identification.

Fullmer and Lillie (1958) found no evidence for the formation of SS or SH reactive sites, - O - SO_4 groupings, or reactive lipid or lipoid structures, after treatment with peracetic acid and suggested that the oxidant might increase the acidity of the fibres which somehow reacted with the aldehyde fuchsin. In a later paper (Fullmer 1960b) examined some of the effects of peracetic acid concluding that this form of oxidation resulted in the formation of end groups utilizable by both proteolytic and mucopolysaccharide enzymes and for aldehyde fuchsin staining. According to Fullmer (1965b) the function of peracetic acid was unknown. Blocking reactions with phenyl hydrazine, methylation, acetylation and deamination failed to diminish the subsequent staining reaction. Furthermore, the oxidation also induced digestibility with commercial elastase and favoured the digestion of the stainable component with β-glucuronidase in an unknown manner. Fullmer et al. (1974) referred to the use of peracetic acid to break the disulphide bonds of insulin but no explanation was offered for its effect upon the oxytalan fibre.

It was claimed by Mander et al. (1968) that periodate oxidation of mouse oxytalan fibres resulted in the formation of enemines which behaved as nucleophilic species in reacting with aldehyde fuchsin.

Moreover, Mander et al. contended that potassium permanganate could be used as a reliable oxidant which was contrary to the experience of Fullmer and Lillie (1958).

Confirmatory investigations of these oxidation studies are not known in the literature. Consequently, the physical and chemical effects of oxidants on the oxytalan fibre remain undetermined.

Staining methods for the oxytalan fibre

Following Fullmer's (1958) demonstration of oxytalan fibres with Gomori's (1950) aldehyde fuchsin, Fullmer and Lillie (1958) detailed the use of this stain and reported that the elastic tissue stains orcein and resorcin fuchsin also identified oxytalan fibres. It was noted that oxytalan fibres were not stained with collagen or reticular stains. The elastic fibre stains orcinol-new fuchsin, Verhoeff's iron haematoxylin, and the Schiff reagent failed to stain oxytalan fibres although rodent elastic fibres reacted with all these stains before and after oxidation. In man, elastic fibres did not react to Schiff reagent. The Hale and Azure A methods were ineffectual both for oxytalan and elastic fibres.

Fullmer and Lillie (1958) and Fullmer (1962) noted that resorcin fuchsin lacked specificity since it stained some oxytalan fibres, but not others. Consequently, Fullmer (1959, 1960a, 1960b, 1961, 1962, 1963, 1965b, 1966b, 1967a, 1967b), Fullmer and Witte (1962) and Fisher and Fullmer (1962) relied upon the aldehyde fuchsin-Halmi and orcein-Halmi stains. Rannie (1963) showed that celestin blue, methylene blue and toluidine blue stained oxytalan fibres. Aldehyde thionin also stained the fibres but proved unreliable. Gawlik and Jarocińska (1964) advocated cresyl violet whereas Mander et al. (1968) used various aldehyde fuchsin dye complexes.

Fullmer (1967b) received the various staining methods for oxytalan fibres and stated that among the elastic tissue stains aldehyde fuchsin was the best since it was the only stain which reliably disclosed all oxytalan fibres. By contrast, resorcin fuchsin and orcein were said to demonstrate fewer fibres. Furthermore, Fullmer reported that oxytalan fibres were readily distinguishable from reticular fibres with several silver impregnation stains. He also stated that peracetic acid-aldehyde fuchsin-Halmi stained neither the precollageneous reticulins, nor supportive stromal reticulins, although the basement membrane between epithelial attachment and subjacent connective tissues was generally well stained.

For light microscope observations Carmichael (1968) modified the application of aldehyde fuchsin and also reported staining oxytalan fibres with chrome alum haematoxylin, and 0.5% basic fuchsin. In 1974, Fullmer et al. described and recommended the use of aldehyde fuchsin as the preferred stain for the demonstration of oxytalan fibres with the light microscope.

Oxytalan fibres in the periodontal ligament of various Australian marsupials were shown by Sampson (1975) to stain well with aldehyde fuchsin, orcein, resorcin-fuchsin and, contrary to the findings of previous investigators, also orcinol-new fuchsin. Bismark brown Y stained the fibres strongly. The addition of 3% acetic acid to a number of elastic tissue stains, particularly alcian blue 8GX, sudan black B, and cresyl fast violet, produced very good staining of oxytalan fibres.

Specificity of oxytalan fibre staining

During their investigations of the oxytalan fibre a number of authors have emphasized that staining with aldehyde fuchsin is not

specific (Fullmer 1959a, 1960a, 1965b, 1967b; Rannie 1963; Löe and Nuki 1964; Gawlik and Jarocińska 1964; Fullmer et al. 1974). Fullmer (1959a, 1959b, 1960b, 1965b, 1967b) and Fullmer and Lillie (1958) noted that aldehyde fuchsin stained not only oxytalan fibres but also reacted with many other substances in the tissues, most of which were mucopolysaccharide in nature. Since the peracetic acid-orcein-Halmi did not react with some of the mucopolysaccharides stained with peracetic acid-aldehyde fuchsin, Fullmer (1959a) considered that orcein was more specific for certain circumstances. Fullmer (1960c) remarked that selective elastic tissue stains were available for the demonstration of elastic tissues, but emphasized that different elastic tissue stains had dissimilar mechanisms of reaction which depended upon different constituents of the fibres. Thus, it was stated that the specificity of the stains could be challenged. Therefore, he suggested that the use of several stains with unlike mechanisms of reaction was indicated for the characterization of elastic fibres. Gawlik (1965) claimed to be able to distinguish elastic, oxytalan and elaunin fibre types using routine histological dyes but Pearse (1968) disagreed with Gawlik's fibre categorization based on elastic tissue staining methods.

Different authors have described the oxytalan fibre as being distinct from collagenous, reticulin and mature elastic fibres (Fullmer and Lillie 1958), an immature or specially modified elastic fibre (Fullmer 1967b; Fullmer et al. 1974), and a fibre exhibiting staining reactions for collagen and keratin but also reacting with nuclear stains (Rannie 1963). The oxytalan fibre has also been characterized as a neural structure (Löe and Nuki 1964), a degenerative form of elastin (Yu and Blumenthal 1967) or degenerating collagen fibres (Selvig 1968). Confusion over the categorization of the oxytalan fibre is understandable since the basis upon which different dyes selectively stain elastic

fibres and oxytalan fibres is the subject of differing opinions.

Various theories have been put forward to explain the staining mechanisms of elastic fibres in general (Ayer 1964; Fullmer 1965b; Horobin and James 1970) as well as the staining chemistry of particular histological dyes used to identify oxytalan fibres including aldehyde fuchsin (Bangle 1954; Mander et al. 1968), resorcin fuchsin (Puchtler, Sweat, Bates and Brown 1961; Puchtler and Sweat 1964; Horobin, Flemming and Kevill-Davies 1974; Meloan and Puchtler 1975) and orcein (Fullmer and Lillie 1956; Fullmer 1959a; Lillie 1969). Bangle (1954) proposed that Gomori's aldehyde fuchsin reacted with aldehyde groups by the mechanism of Schiff base formation; a veiw endorsed by Mander et al. (1958). Moreover, as a result of their investigations, Mander et al. concluded that important chemical differences existed between elastic and oxytalan fibres.

Fullmer (1965b, 1967b, 1969) reported that aldehyde fuchsin was a transitory dye formed at room temperature from basic fuchsin and paraldehyde in an acidic alcoholic solution. He stated that effective concentrations of the stain in the mixture were present for about 1 week and thereafter the staining solution should be discarded. Mander et al. (1968) disagreed, claiming that aldehyde-fuchsin was an effective stain for oxytalan fibres for eight weeks or more after preparation.

Different investigators have demonstrated that elastic stains are not specific for the protein elastin but can be made to stain collagen (Fullmer and Lillie 1957; Puchtler et al. 1961; Puchtler and Sweat 1964) and keratin (Rannie 1963). Ayer (1964) and Pearse (1968) have reviewed the diverse opinions regarding the mechanisms associated with the staining of elastic fibres by various types of elastic dyes.

Pearse pointed out that the mechanism by which most elastic stains worked remained an enigma and that the staining of elastic tissue with a particular dye might be due to other components than the characteristic protein elastin. Furthermore, he stressed that histological staining of elastic fibres suggested that there were wide differences in the elastic fibres from different tissues. Despite the fact that these differences were unexplained, Pearse considered they were most probably of a physical nature.

Although Fullmer (1959b, 1960a, 1960b, 1961, 1965b, 1966b, 1967b), Fisher and Fullmer (1962), Hamner and Fullmer (1966) and other investigators have used the term histochemical to describe the specificity of the stains and staining techniques used to identify oxytalan fibres, such terminology has been questioned. The use of the term histochemical has been considered at length by many authors including Glick (1967) and Pearse (1968). Ross (1973) repudiated the concept that histological stains such as Weigert's resorcin fuchsin or orcein used for the identification of elastic fibres were histochemical in nature. He stated that the means by which the various histological dyes appeared to selectively stain elastic fibres was unknown and consequently their use could not be attributed to a histochemical response. Ross considered that these dyes appeared to bind selectively to some component of the elastic fibre and therefore "histoalchemy" was a more appropriate term to describe their response.

A recent concensus of opinion as to the staining of oxytalan fibres was reported by Fullmer et al. (1974) who stated that oxytalan fibres were selectively, but not specifically, stained with the peracetic acid or Oxone-aldehyde fuchsin method. Furthermore, these authors remarked that in interpreting the staining reaction it must be

recognized that the violet aldehyde fuchsin-stained material was the consequence of a differentiation step in which the stained structures were those that persisted after immersion in a 70% alcohol differentiation bath. They also stressed that current histological staining methods were unable to discriminate between immature or developing elastic tissues and oxytalan fibres. Over 20 years ago Fullmer and Lillie (1956) stated that their investigations indicated that various elastic tissue stains had different mechanisms of reaction and probably different reactive end groups.

The function of the oxytalan fibre system

The few investigators who have attributed a specific function to these fibres have usually advanced differing theories. Fullmer (1959b, 1960c, 1964, 1965a, 1965b, 1967a, 1967b) noted that the fibres increased in number and size with accentuated functional demands. He observed that the human periodontal ligament was characterized by the customary absence of elastic fibres and the presence of oxytalan fibres. When elastic and oxytalan fibres were observed in the periodontal ligament they were found to be associated with blood and lymph vessels (Fullmer 1959b, 1962, 1967a; Fisher and Fullmer 1962; Kohl and Zander 1962; Soule 1967).

Fullmer (1960b) declared that oxytalan fibres were present in connective tissues that required little or no elasticity. He suggested that in sites such as the periodontal ligament and tendons characteristic elastic tissue was not required, or could actually be detrimental to function and, therefore, an altered or incomplete elastic structure was formed. In 1962 Fullmer stated that although the function of the oxytalan fibre was unknown he presumed that the fibre provided elasticity and a suspensory function within the periodontal membrane.

Several investigators have ascribed a particular function to the oxytalan fibre. Rannie (1963) assumed that the fibres added strength to the periodontal ligament and proposed that they might have some anchoring effect which would impede overstretching of the tissue and so prevent ischaemia due to obliteration of the vascular channels.

Löe and Nuki (1964) precluded any tooth-supporting function by oxytalan fibres and claimed that they were natural elements. Goggins (1966) disputed Löe and Nuki's neural concept but offered no alternative suggestion as to the function of the fibre.

Simpson (1967) considered that the fibres enhanced the stability of the tooth in its socket and assisted in tooth retention.

Fullmer (1967a, 1967b) stated that the function of oxytalan fibres had not been precisely determined and suggested that a mechanism existed for their increase in size. Soule (1967) noted that the fibres were not seen until developing crocodilian teeth came into function. He claimed that these findings lent support to Fullmer's (1958) opinion that the fibres appeared in areas of stress.

Carmichael (1968) suggested that oxytalan fibres were concerned with the stability and possible patency of the periodontal vessels under pressure permitting them to accommodate to distortional and compressive strains while maintaining their patency.

Because of the so-called elastic nature of oxytalan fibres Edwards (1968) suggested that they might contribute to the relapse tendency of orthodontically rotated teeth. A further opinion was introduced by Selvig's (1968) proposal that oxytalan fibres were partially decomposed collagen fibrils. Brain (1969) stated that the express function of oxytalan fibres was unknown.

Boese (1969) suggested that oxytalan fibres would be stretched

during orthodontic rotation and would, therefore, contribute to posttreatment relapse. Edwards (1970) speculated that surgical detachment of the elastic-like oxytalan fibres would significantly alleviate relapse following human tooth rotation. In a later paper Edwards (1971) implied that oxytalan fibres contributed to orthodontic relapse. He suggested that the surgical removal of excess gingivae and their oxytalan fibres between teeth approximated across an extraction space would alleviate reopening of the extraction space in orthodontic patients.

Parker (1972) was unable to indicate a specific function for these fibres but suggested that their role was secondary and complementary to that of collagen. He proposed that oxytalan fibres acted as a safeguard against abnormal forces causing separation and destruction of tissues. It was also stated by Hurst (1972) that the function of the oxytalan fibre in the periodontium was unknown. However, he made the interesting observation that the onset and progression of ankylosis in reimplanted monkey teeth was accompanied by a reduction in the size and number of oxytalan fibres present in the periodontal ligament and to a lesser degree in the transseptal region.

In their review of the oxytalan fibre Fullmer et al. (1974) reiterated that the precise function of the fibre remained undetermined. The suggestion was made, however, that fibre size and number was a physiological response to the demands of functional stress. Oxytalan fibres were said to be definitely tooth orientated and at least some of the fibres were thought to support blood and lymphatic vessels.

A totally different concept was put forward by Beertsen et al. (1974) who hypothesized that oxytalan fibres functioned as a guiding and supporting system for the eruption of the rodent incisor.

Campbell et al. (1975) suggested that stretched oxytalan fibres exhibited elastic rebound and therefore contributed to the reopening of orthodontically corrected midline diastemas.

Soames and Davies (1975) proposed that oxytalan fibres might help to stabilize the blood vessels and epithelial attachment under functional pressure. Markens and Oudhof (1976), however, doubted that oxytalan fibres had a mechanical function although they reported an increase in fibre numbers as teeth were exposed to greater occlusal loading.

At the present time the function of the oxytalan fibre is imperfectly understood. Little attention has been given to this important aspect of a unique fibre which is especially associated with connective tissues subject to stress.

In order to clarify the operational role of this fibre system this series of investigations was undertaken to:

1. Establish a model of oxytalan fibre distribution in the periodontium.
2. Demonstrate that the oxytalan fibre is metabolically and structurally distinct from collagen.
3. Examine the response of oxytalan fibres to different types of tooth loading in the animal periodontal ligament.
4. Compare the oxytalan fibre systems in the animal and human periodontal tissues.

CHAPTER II

THE OXYTALAN FIBRE IN THE MOLAR PERIODONTAL LIGAMENT OF THE NORMAL MOUSE MANDIBLE

Human, mouse, rat and guinea pig periodontal ligament was initially included in the connective tissues shown to contain oxytalan fibres (Fullmer 1958; Fullmer and Lillie 1958). Thereafter, oxytalan fibres were demonstrated in the periodontal ligament of developing humans, infants and adults (Fullmer 1959a, 1959b, 1960a, 1960b, 1961, 1966a, 1966b, 1967a, 1967b; Fullmer et al. 1974; Rannie 1963; Löe and Nuki 1964; Simpson 1967; Griffin and Harris 1967; Harris and Griffin 1967; Provenza et al. 1967; Edwards 1971; Roche 1972; Fullmer et al. 1974), monkeys (Fullmer 1960b; Kohl and Zander 1962; Rannie 1963; Löe and Nuki 1964; Boese 1969; Parker 1972; Hurst 1972) and dogs (Fullmer 1960b; Löe and Nuki 1964; Edwards 1968; Soames and Davies 1975).

Other investigations described the fibres in the periodontal ligament of cattle, the rabbit, deer, sheep and swine (Fullmer 1960b), mice (Fullmer 1960b, 1966b; Beynon 1967; Carmichael 1968; Mander et al. 1968), rats (Gardner 1960; Fullmer 1960b; Kennedy and Kennedy 1963; Sheetz et al. 1973), the Caiman and Alligator (Soule 1967), the Trigger-fish (Soule 1969), and various Australian marsupials (Sampson 1975) which possessed marked differences in their dentitions and a variety of temporo-mandibular articulations. Oxytalan fibres have also been reported in the vole (Oxberry 1975), guinea pigs (Fullmer 1958) and

the mini-pig (Markens and Oudhof (1976).

Thus the presence of the oxytalan had been demonstrated in a wide variety of species. For the present series of investigations an animal had to be selected which was suitable for a variety of experimental purposes.

MATERIALS AND METHODS

Choice of the mouse as the experimental animal

According to Fullmer (1966b) the electron microscopic appearance of the fibres in man and rat is indistinguishable. Furthermore, he stated (Fullmer 1967b) that the distribution of oxytalan fibres in the periodontium of mice, rats and monkeys approximates that of man.

Accordingly, the rat was selected as an appropriate laboratory animal for the commencement of this investigation. Rats had the advantage of being in plentiful supply and it was considered that the three mandibular molars on each side of the jaw would provide a small, functionally independent group of teeth for examination of the periodontal ligament. However, tracing of the oxytalan fibre system within the molar periodontal ligament proved to be difficult. Therefore, the albino mouse was evaluated as an alternative animal. Histological examination of the molar periodontal ligament in the mouse mandible revealed that the oxytalan system was more readily identified and traced after appropriate staining.

Mice also possessed the advantages of being readily available, inexpensive to obtain and feed, a large number could be housed in a small space, they were easily handled and readily anaesthetized by

intraperitoneal injection. Furthermore, the molars had bifurcated and not trifurcated roots as in rats while the number of paraffin sections required for a complete series through the approximating molars in a particular plane was approximately one third as many as for the rat.

The first 20 mice used were albino males and females of the CBA strain bred at the Institute of Medical and Veterinary Science, Adelaide, South Australia. Thereafter, the remaining 70 experimental animals came from the Waite Agricultural Research Institute of the University of Adelaide. Mice were killed by ether inhalation in a glass dessicator.

Histological Fixation

Different fixatives have been used for the various animal and human tissues shown to contain oxytalan fibres. Fullmer has generally employed Lillie's (1965) aqueous neutral calcium acetate formalin for 18 to 24 h at 25^{o}C (Fullmer and Lillie 1958; Fullmer 1959a, 1959b, 1960b, 1960c, 1961, 1966b; Fullmer and Witte 1962), although 95% alcohol for 24 h (Fullmer and Lillie 1958; Fullmer 1959a, 1959b, 1961) and 70% alcohol (Fullmer 1961) have proved to be satisfactory. Carnoy (Fullmer 1961; Oxberry 1975), Orth's and Zenker's solutions (Gawlik and Jarocińska 1964), 10% formalin (Fullmer 1960a; Fisher and Fullmer 1962; Gawlik and Jarocińska 1964; Goggins 1966; Kanouse 1966; Hamner and Fullmer 1966; Edwards 1968; Hurst 1972) and 10% buffered formalin (Löe and Nuki 1964; Baratieri 1967; Parker 1972) provided fixation in several studies.

Sampson (1975) employed 10% neutral buffered formalin for marsupial tissues whereas Soames and Davies (1975) fixed with a glutaraldehyde-paraformaldehyde mixture. Rannie (1963) failed to state

the type of fixative used while other investigators provided minimal information such as alcohol (Gawlik and Jarocińska 1964), formalin (Gawlik 1965), formol-calcium (Carmichael and Fullmer 1966), neutral formalin (Soule 1967), and buffered formalin (Carmichael 1968).

Fullmer et al. (1974) recommended Lillie's (1965) aqueous neutral calcium acetate formalin for 24 h at 25°C. It was reported (Fullmer 1967b) that formalin and alcohol fixed oxytalan fibres were unsuitable for some enzymatic hydrolysis tests while Fullmer et al. (1974) have warned that fixatives containing chromates or mercury should not be employed as they interfered with the staining reaction.

Preliminary studies in the present investigation revealed that 10% neutral buffered formalin and Lillie's (1965) neutral calcium acetate formalin solution were suitable fixatives for oxytalan structures in the mouse mandible. Immediately after sacrifice mouse heads were placed in 10% neutral buffered formalin or Lillie's aqueous neutral calcium acetate formalin (Appendix I) for a minimum of 24 h at 25°C. The mandibles were then carefully dissected out, freed of all adhering soft tissue apart from the attached gingivae around the teeth, and divided at the symphysis. A small number of maxillae including the three molars with their attached periodontal tissues were fixed similarly.

Demineralization

For animal, and also human tissues, 5% formic acid has been the choice of the majority of investigators (Fullmer and Lillie 1958; Fullmer 1959a, 1959b, 1960a, 1960b, 1960c, 1961, 1966a; Fullmer and Witte 1962; Löe and Nuki 1964; Hamner and Fullmer 1966). Other demineralization techniques used in oxytalan fibre studies included electrolytic demineralization with a solution of formic and

hydrochloric acid (Goggins 1966), 3% hydrochloric acid (Kanouse 1966), 10% trichloracetic acid (Soule 1967), 5% trichloracetic acid (Soule 1969), and buffered ethylene diamine tetra-acetic acid (EDTA) in formalin at 37°C (Carmichael 1968), and buffered 10% EDTA (Oxberry 1975).

Mander et al. (1968) employed 10% EDTA in 10% formaldehyde for 3 to 4 d. Superficial demineralization with EDTA was carried out by Soames and Davies (1975) before preparing paraffin embedded blocks. Edwards (1968) and Hurst (1972) used an unspecified combination of formic acid and sodium citrate. Rannie (1963) and Edwards (1971) provided no information on their demineralization methods and Parker (1972) merely stated that he used formic acid. Fullmer et al. (1974) advocated the 10% EDTA method of Fullmer and Link (1964) or 5% formic acid.

During the initial stages of the present investigation a few mandibles were demineralised with EDTA (Fullmer and Link 1964). The results were very satisfactory but financial and equipment considerations necessitated using alternative methods which proved to be most successful for histologic studies.

Mandibles were demineralized in 5% formic/formate solution (Appendix I) changed every 24 h. The mandibles were washed in running water for 2 h. Some jaws were processed in Decal (Omega Chemical Co., New York, United States) which reduced the time from approximately 96 h to 3 or 4 h but this method did not prove satisfactory as a routine procedure (Appendix I).

Neutralization

Upon completion of decalcification, mouse specimens were placed in 5% sodium sulphate for 12 h.

Tissue Processing

The specimens were prepared for paraffin wax blocking by the Double Embedding Method (Appendix I), and paraffin blocked on a Tissue Tek II Tissue Embedding Centre (Lab-Tek Products, Naperville, United States). The small size of the mouse mandible made it difficult to obtain the desired orientation of a particular tooth when blocking in wax. Because of the progressive lingual axial inclination from the first to the third molar it was rarely possible to orientate the axial plane of section through more than one tooth.

Several methods were tested to provide the required axial inclination of a particular molar. Methods included:

(i) the placement of small spots of acrylic artist's colours at different sites corresponding to the axial mesiodistal plane of a molar. Alignment of the colours during blocking and sectioning often aided orientation but proved unreliable for routine purposes.

(ii) trimming the lingual side of the mandible alongside the molars with a Gem blade to provide a flat surface parallel to the observed axial mesiodistal plane of the first molar. This preparation was made after demineralization but before the specimens were placed in salicylate.

(iii) use of a fine reamer inserted into the first molar after fixation. The mesial pulpal horn of the molar was exposed and 1.0 mm of a No. 12, 28 mm stainless steel hand broach reamer (Produits Dentaires, S.A. Vevey, Switzerland) threaded into the root canal. Each mandible was processed on the reamer which was used to correctly orientate the specimen during paraffin blocking. For buccolingual or horizontal axial sections a

straight wire was attached to the condyle with a cyanoacrylate adhesive to achieve the desired orientation when blocking. Occasional buccal or lingual deviation of the mesial root and difficulties in the management of tissues during processing limited the usefulness of this procedure.

For routine purposes trimming alongside the lingual alveolus of a particular molar, as in (ii), produced the most reliable guide to specimen orientation.

Histological sectioning

Most studies have used sections cut at six to eight μm (Fullmer and Lillie 1958; Fullmer 1959a, 1959b, 1960a, 1960b, 1960c, 1961, 1966a; Fisher and Fullmer 1962; Fullmer and Witte 1962; Hamner and Fullmer 1966; Kanouse 1966; Edwards 1968; Hurst 1972). Parker (1972) cut his material at 5 μm whereas Rannie (1963), Löe and Nuki (1964) and Goggins (1966) did not specify the thickness of their sections. However, as indicated by Sheetz et al. (1973) and Fullmer et al. (1974) the optimal tissue thickness is dependent upon the needs of the investigation.

Mandibles from 90 mice were equally allocated to three groups for serial sectioning in the mesiodistal, buccolingual, or horizontal planes to the vertical axis through the first molar using an ERMA (Japan Optical Works, Tokyo, Japan) or LEITZ (E. Leitz, Wetzlar, West Germany) rotary microtome. Sections were usually cut at 8 μm although this thickness was varied from 5 to 10 μm. The ribbons were floated on a warm water bath at 45°C, transferred to clean glass slides, and placed in a hot air oven at 60°C for 1 h.

Oxidation of Sections

Many studies have preoxidised tissue sections with Greenspan's (1950) peracetic acid (Fullmer 1958, 1959a, 1959b, 1960a, 1960b, 1960c, 1961, 1966a, 1967b; Fullmer and Lillie 1958; Fullmer and Witte 1962; Löe and Nuki 1964; Gawlik and Jarocińska 1964; Gawlik 1965; Goggins 1966; Fullmer 1966a; Kanouse 1966; Baratieri 1967a; Soule 1967; Hurst 1972). Successful staining has also been reported with other oxidizing agents such as performic acid, potassium permanganate, bromine (Fullmer and Lillie 1958; Fullmer 1967b) or a weak 0.2% solution of potassium permanganate at pH 2.0 (Carmichael 1968; Mander et al. 1968).

Rannie (1963) introduced a 10% aqueous solution of Oxone at 25°C as a safe, less caustic, easy to use, and improved oxidant in lieu of peracetic acid. Other investigators who used Oxone included Simpson (1967), Carmichael (1968), Mander et al. (1968) and Edwards (1968, 1971).

Fullmer and his associates (Fullmer 1966a, 1967b; Carmichael and Fullmer 1966; Sheetz et al. 1973; Fullmer et al. 1974) declared that Oxone was the preferred oxidant as it did not cause tissue destruction. Generally, Oxone treatment has been advocated in a 10% aqueous solution for 1 h at 25°C (Fullmer 1966a, 1966b; Sheetz et al. 1973; Soames and Davies 1975). Carmichael (1969) advised the use of aqueous Oxone approximating a 10% concentration at room temperature for 15 min. In 1969 Fullmer (personal communication) recommended the application of Oxone for 60 min. However, Fullmer et al. (1974) advocated the use of fresh 10% aqueous Oxone for 30 to 60 min.

The effectiveness of Oxone was demonstrated by substituting potassium permanganate with 10% aqueous Oxone for 10 min in the Gordon and Sweet silver stain. This technique normally did not identify

oxytalan fibres but the use of Oxone resulted in their clear demonstration in the mouse periodontal ligament.

Because of its proven advantages Oxone was used throughout the present study. Tests using different concentrations, and times from 10 to 120 min indicated that for mouse tissues in particular, the most suitable oxidation method was a fresh 10% aqueous solution of Oxone for 30 min at 37^{o}C for each batch of 6 slides. Oxidation beyond one hour frequently resulted in excessive background staining with aldehyde fuchsin.

Histological Staining

Fullmer and Lillie (1958) detailed the preparation and use of aldehyde fuchsin and reported that the elastic tissue stains orcein and resorcin fuchsin also identified oxytalan fibres. These authors reported that resorcin fuchsin lacked specificity since it stained some oxytalan fibres, but not others. Consequently, Fullmer (1959a, 1959b, 1960a, 1960b, 1960c, 1961, 1962, 1966a), Fullmer and Witte (1962) and Fisher and Fullmer (1962) relied upon aldehyde fuchsin-Halmi and orcein-Halmi stains to identify oxytalan fibres in oxidised tissue sections.

Fullmer (1967b) reviewed the various staining methods and stated that among the elastic tissue stains aldehyde fuchsin was the best method for oxytalan fibres since it was the only stain which reliably stained all oxytalan fibres. By contrast, resorcin fuchsin and orcein demonstrated fewer fibres. He also stated that the peracetic acid-aldehyde fuchsin-Halmi stain which identified oxytalan fibres stained neither the precollagenous reticulins, nor supportive stromal reticulins although the basement membrane between epithelial attachment and subjacent connective tissues was generally well stained.

In 1974, Fullmer et al. described and recommended the use of aldehyde fuchsin as the preferred stain for the demonstration of oxytalan fibres at the light microscope level.

Application of the dyes celestin blue, methylene blue, toluidine and aldehyde thionin (Rannie 1963) did not reveal mouse oxytalan fibres. In the present study the oxytalan fibres occasionally stained lightly with aldehyde fuchsin after prior treatment with glacial acetic acid, or by direct exposure to aldehyde fuchsin. The reasons for these staining discrepancies remained undetermined.

The comparative merits of aldehyde fuchsin, orcein and resorcin fuchsin (Fullmer and Lillie 1958) were evaluated on mouse tissue and the preference of Fullmer (1967b) for aldehyde fuchsin was confirmed. Subsequently, Fullmer et al. (1974) have reaffirmed this preference.

Certain conditions proved to be important for the reliable use of aldehyde fuchsin with mouse tissues. Firstly, the selection of crystalline basic fuchsin with a metallic sheen (Sweat, Puchtler and Woo 1964) and secondly, the use of freshly manufactured paraldehyde (Fullmer 1969; Moment 1969) for the weekly preparation of Gomori's (1950) aldehyde fuchsin. Old paraldehyde made the aldehyde fuchsin stain unreliable or ineffectual. As stated by Fullmer (1959b, 1960b, 1961, 1967b), Fullmer and Witte (1962) and Fullmer et al. (1974) the correct laboratory schedule in the preparation, ageing, use, and the discarding of aldehyde fuchsin solution after one to two weeks was an essential prerequisite for successful fibre staining.

Early in the investigation modifications were made to the staining techniques to assist identification of the fine fibres of the oxytalan meshwork and enhance photographic recording. Lillie-Mayer's

alum haematoxylin was omitted from the aldehyde fuchsin stain (Fullmer and Lillie 1958; Fullmer et al. 1974) to minimize cellular staining and the recommended Halmi counterstain was replaced with light green SF (Appendix II) to improve fibre image contrast for black and white negatives. As reported by Fullmer and Lillie (1958) and Fullmer (1959a, 1967b) the aldehyde fuchsin introduced considerable background staining in sections because it stained the mucopolysaccharide substances in the connective tissues in addition to the oxytalan fibres. This difficulty was minimized in several ways including the use of fresh aldehyde fuchsin, (Fullmer et al. 1974) and/or treatment of stained sections with 4% hydrochloric acid in 70% alcohol (Löe and Nuki 1964).

Oxytalan fibres were demonstrated by oxidizing every fifth section with Oxone and then staining with modified aldehyde fuchsin-light green SF. Orcein-Halmi (Fullmer 1959) or Weigert's resorcin fuchsin (Lillie 1965; Fullmer and Lillie 1958) were employed when necessary as confirmatory stains. Adjacent control sections were stained simultaneously without preoxidation. Staining every fifth section permitted a comprehensive examination of each mandible and left sufficient sections for additional investigation where regions of special interest were evident.

Elastic fibres in the periodontal tissues were demonstrated in unoxidized sections with aldehyde fuchsin (Fullmer and Lillie 1958), Weigert's resorcin fuchsin, Verhoeff's iron haematoxylin, Taenzer-Unna orcein (Lillie 1965), and orcinol-new fuchsin (Fullmer and Lillie 1956). Collagen was identified with van Gieson (Lillie 1965) and 0.02% light green SF. Reticulin was stained with the silver impregnation methods of Gordon and Sweet (Pearse 1968), Naoumenko and Feigin (1974) and Lillie's allochrome (Lillie 1965). Nerve fibres were

revealed with the Bielschowsky, Bodian, and Hirano-Zimmerman methods (Luna 1968) and referred to in Appendix II.

Microscopy and Photography

All sections were examined with an Olympus (Olympus Optical Co. Ltd, Tokyo, Japan) model FHT-533 microscope. Scanning records taken on Polaroid (Polaroid Corporation, Cambridge, United States) black and white Land Pack Film, Type 107, using an Olympus PM-10-A photomicrographic camera with adapters.

Black and white negatives were obtained with 102 x 127 mm Ilford (Ilford Limited, Basildon, England) FP4 cut sheet film using a Zeiss (Carl Zeiss, Oberkochen, West Germany) Axiomat microscope or a Leitz (E. Leitz, Wetzlar, West Germany) Ortholux microscope. A Zeiss green interference filter 46 78 07 provided enhanced image contrast of oxytalan fibres. Negatives were developed in Ilford ID2 according to manufacturer's instructions, and printed on Ilford Ilfobrom or Kodak (Eastman Kodak Company, Rochester, United States) Veribrom paper. Colour transparencies were taken on Kodak 35 mm Photomicrography Film 2483 developed by the E-4 process.

FINDINGS

In all sections the oxone-aldehyde fuchsin-light green technique produced consistent and well-defined staining of oxytalan fibres in the periodontal ligament of molar and incisor teeth. Oxytalan fibres were not stained with routine elastic, collagen or reticular stains. The elastic stains orcinol-new fuchsin, Verhoeff's iron haematoxylin, and the Schiff reagent also failed to stain the fibres.

The arrangement of the oxytalan fibre system to be described

in the periodontal ligament of mandibular molars corresponded in essential detail with the system observed in the periodontium of maxillary molars and the continually erupting mandibular incisor.

Mesiodistal Sections

Oxytalan fibres extended from the dentinocemental junction into the periodontal ligament where they became thicker and curved apically to enter the walls of vessels adjacent to the alveolar bone. Oxytalan fibres also linked the vessels of the periodontal ligament to each other. Where vessels were orientated occluso-apically the adjacent oxytalan fibres were more numerous forming dense aggregations of fibres which were designated oxytalan fibre "tracts". At the cementum surface oxytalan fibres were distributed as a fine plexus which enveloped the root and related the larger oxytalan fibres to each other where they inserted into the cementum (Figure 2.1).

Major oxytalan fibres were arranged in a predominantly occluso-apical orientation with a finer supplementary system of interconnecting fibrils. Continual branching and communication between the oxytalan fibres resulted in the formation of a dense three-dimensional oxytalan fibre meshwork. This meshwork which extended from the gingival to the apical region of the periodontal ligament weaved between the collagen fibres and associated the tooth with the various vessels within the ligament (Figure 2.2). Adjacent to the cemento-enamel junction some oxytalan fibres curved occlusally toward blood vessels of the interdental gingival papilla.

The meshwork extended across the crest of each interproximal alveolar septum as a continuous system which joined adjacent teeth and their enveloping oxytalan fibre meshworks. At the mesial and distal aspects of the first and third molars, respectively, the oxytalan fibre

meshwork terminated above the alveolar crest adjacent to the tendinous insertions of masticatory muscles with their clearly defined condensations of elastic fibres. Oxytalan fibre diameter varied from the limit of resolution of the light microscope to 0.8 μm. Some of the largest oxytalan fibres occurred opposite the coronal third of the interseptal bone. These fibres had a pronounced ribbon-like appearance being relatively broad and thin.

Large vessels which penetrated the alveolar wall apical to the middle of the root lost all staining evidence of their accompanying oxytalan fibres upon entering the bone. Similarly, vessels which passed through lateral canals between the periodontal ligament and the pulp chamber also failed to show staining of oxytalan fibres after entering the root.

Areas of resorption involving the dentine were sometimes present in the cervical and middle third of the root. Repair of these areas with cementum was accompanied by the formation of new oxytalan fibres extending from the cementum to nearby vessels (Figure 2.3).

When the oxytalan fibre meshwork was cut obliquely to the principal occluso-apical orientation, the cut ends of the fibres appeared turned over as if they had been stretched prior to severance by the microtome knife and then recoiled like an elastomeric substance. Rotation of the tissue block through 90^{o} in the microtome chuck resulted in a comparable change in the angle of the curved ends of the severed fibres.

Orientation and distribution of the oxytalan fibre meshwork in mesiodistal sections demonstrated five distinct regional patterns (Figure 2.4). Four patterns were characteristic of the corresponding

regions of the periodontal ligament of each molar tooth. The fifth pattern was observed only at the mesial and distal extremities of the molar segment.

Region A

The fibres arose at the dentinocemental junction and occasionally branched as they passed through the cementum toward the periodontal ligament. Where the fibres emerged from the cementum, they were almost at right angles to the surface. The fibres contributed to the formation of several main fibre groups shown diagrammatically in Figure 2.5.

1. A delicate intercommunicating plexus alongside the cementum.
2. Fibres curving upward toward the basement membrane of the interdental papilla.
3. Horizontal fibres dividing and anastomosing across the transseptal region to communicate with similar fibres extending from the approximating tooth.
4. Fibres which, after emerging from the cementum, increased in diameter as they changed direction to an occluso-apical orientation and extended apically.
5. A prominent fibre group that curved over the interseptal alveolar crest and established continuity between Group 4 fibres of adjacent teeth. This arch of fibres generally was separated from the alveolar surface by a region almost devoid of oxytalan staining elements. From the superior aspect of the arch, clearly defined fibres travelled occlusally and terminated just below the basement membrane.
6. Fibres observed in cross-section and located between the basement membrane and the superior aspects of the arch. These fibres

passed from the buccal to the lingual sides of the gingival papilla as they encircled the crown or traversed the papilla.

In Region A certain oxytalan fibres attained their maximal diameter in three places: within the cementum near the cementoenamel junction; the site where the fibres became occluso-apically orientated; and in the complex arched network above the interseptal bone. The direction of the oxytalan fibres in Region A did not always conform with that of the principal collagen bundles. Oxytalan fibres in Groups 4 and 5 were orientated predominantly at right angles to the alveolar crest and to the horizontal collagen fibres. The intercommunicating plexus also differed from that of the local collagen arrangement. Only Groups 2, 3 and 6 were orientated similarly to the collagen bundles.

Canals containing vessels and their accompanying oxytalan fibre system penetrated the interseptal bone in an anteroposterior direction providing direct communication between the periodontal ligament of adjacent teeth. These shunts in the oxytalan fibre meshwork were always supplementary to the other oxytalan fibre groups routinely straddling the interseptal crest (Figure 2.6).

Region B

After the fibres emerged from the cementum and contributed to the plexus formation, they increased in diameter and assumed an occluso-apical orientation between the principal collagen bundles aligned obliquely to them. Fibres that appeared to be discrete at low magnification often were shown to continually divide and reunite along their length and to give off subsidiary branches to adjacent fibres when observed under high magnification. The vertical oxytalan fibres adjoining blood vessels also aggregated to form well-defined

fibre tracts with fine interconnecting branches. The main fibres and fibre tracts extended toward more apically positioned vessels adjacent to the alveolar walls, or below the root apex (Figure 2.7). This arrangement resulted in a predominantly vertically orientated interlacing fibre meshwork enclosing each root and linking the blood vessels occluso-apically.

Oxytalan fibre shunts accompanied vessels traversing anteroposterior canals in the occlusal portion of this region of the interseptal bone. As the bone widened apically vessels within canals, especially where marrow spaces interposed, were not accompanied by oxytalan fibre shunts.

Region C

Adjacent to the junction between Regions B and C the major oxytalan fibres curving apically from the cementum into the periodontal ligament formed a less acute angle with the cementum than elsewhere on the root surface opposite the alveolar wall. These fibres united with the vertically arranged portion of the oxytalan system which changed direction and curved beneath the root apex to form a fine irregular meshwork. The fibres were distributed among the numerous vessels present and within their walls (Figure 2.8). As in the rest of the periodontal ligament, the oxytalan meshwork in the apical region received numerous additional fibres from the adjacent cementum.

The orientation of the oxytalan system in the apical region was predominantly at right angles to the generally accepted arrangement of the collagen fibres. Where a major vessel penetrated the alveolar wall, the accompanying oxytalan structures occasionally appeared to be embedded in the alveolar bone. Isolated oxytalan fibres were sometimes observed within the regions of hypercementosis surrounding the molar

apices (Figure 2.7). These fibres had the characteristic orientation of the largest oxytalan fibres in the apical region of the periodontal ligament and were similar in size. In some mice, the mandibular nerve came into contact with the apical periodontal ligament surrounding the mesial root of the first molar. Fibres of the oxytalan meshwork did not demonstrate a preferential orientation toward the nerve as they did for nearby blood vessels. Oxytalan fibres appeared to curve round and bypass the capsule of the nerve.

Region D

In the interradicular region, numerous oxytalan fibres extended downward from the cementodentinal junction to the walls of blood vessels which frequently occupied more than half the width of the periodontal ligament. A few vessels passed apically into the crest of the alveolar bone losing all evidence of their accompanying oxytalan fibres after the vessels entered the bone. As a contrast, where vessels traversed horizontal canals in the interradicular bone just below the crest, the oxytalan fibres accompanied the vessels throughout their length and thus formed a direct connection between the oxytalan fibre meshwork on either side of the interradicular bone. Across the interradicular crest the fibres of the oxytalan meshwork, in addition to showing a vertical orientation, also demonstrated a random distribution between the irregularly arranged vessels. Fibres that extended across the interradicular crest were less regularly arranged than those of the arch formation that straddled the interseptal bone.

Toward the lateral boundaries of the periodontal ligament in Region D the oxytalan fibres assumed the vertical arrangement alongside the roots characteristic of Regions B and C. Anteroposterior

canals containing vessels and oxytalan fibre shunts were present in the interradicular crest.

Region E

On the mesial side of the first molar and distal side of the third molar the oxytalan fibres passed above the crest of the alveolar bone and terminated adjacent to condensations of elastic fibres associated with nearby muscle attachments. The masseter muscle demonstrated a tendinous extension into the cervical portion of the periodontal ligament on the mesial aspect of the first molar. For unknown reasons histological identification of this feature with the aldehyde fuchsin stain was enhanced by placing the mice on a lathyritic diet for 1 week (Figure 2.9).

Tangential Sections: buccal and lingual root surfaces

Sections in the mesiodistal plane revealed the vertical orientation of the oxytalan fibre meshwork extending from the basement membrane of the gingival papilla and the cervical portion of the cementum to the apical region of the teeth. The meshwork consisted of three principal fibre arrangements. There were the major oxytalan fibres which extended long distances continually dividing and reuniting with the branches of similar nearby fibres. Adjacent to occluso-apically orientated blood vessels these major oxytalan fibres tended to aggregate forming dense collections of fibres called tracts. Between the vertically arranged fibres was a very fine network of subsidiary interlacing fibres which linked each other and the larger fibres together forming a meshwork with a predominant occluso-apical orientation which weaved between the collagen fibre bundles (Figures 2.10 and 2.11).

The principal vessels in the periodontal ligament also had

an occluso-apical alignment and interconnected by means of lateral anastomosing branches. Where these communicating branches were curved, the vascular system formed eliptical-shaped arrangements (Figure 2.12). Oxytalan fibres extended from the basement membrane of the gingivae to aggregate around the vertically orientated vessels to form fibre tracts or course within the walls of the vessels parallel to their long axis (Figure 2.13). Those oxytalan fibres which passed around or through the walls of the communicating vessels generally maintained their occluso-apical direction irrespective of the curvature of the vessel or its alignment to the occlusal plane. The communicating vessels were linked vertically to each other by the oxytalan fibre meshwork and also related to the tooth by oxytalan fibres which extended between the cementum and the vessels (Figure 2.14).

Sections tangential to the buccal or lingual surface of the molars revealed the existence of a crossover arrangement of the fibres in the oxytalan meshwork. Crossovers were observed in serial preparations opposite the junction of the middle and apical thirds of the root (Figures 2.15 and 2.16). In the cervical region oxytalan fibres curved outwards from the cementum surface and ran horizontally around the tooth to contribute to a circular plexus which extended into the gingival region and was particularly prominent interproximally.

Buccolingual Sections

The orientation and distribution of the oxytalan fibre meshwork demonstrated five regional patterns which corresponded with Regions A, B, C, D and E defined in the mesiodistal sections.

Region A

Immediately above the alveolar bone was a zone generally

devoid of oxytalan fibres. Occlusal to this zone the prominent oxytalan fibres of the group arching mesiodistally over the alveolar crest were seen in cross-section as small dots. Above these fibres the finer oxytalan fibres of the transseptal group could be identified. The most clearly defined oxytalan fibres were those passing in a buccolingual direction and located between the transseptal oxytalan fibres and the basement membrane of the interdental papilla. These particular fibres were also associated with the circular oxytalan fibre group seen in these sections through a segment of their perimeter as fine horizontal fibres. Some oxytalan fibres were observed extending occlusally from the Group 5 fibres illustrated in Figure 2.5 to the basement membrane of the interdental epithelium. On the buccal side of the alveolar crest the masticatory muscles attached to the periosteum adjacent to the crest and the gingival tissues.

Region B

On both the buccal and lingual aspects of all molar roots the oxytalan fibre arrangement and distribution was similar to that observed in mesiodistal sections. After emerging from the cementum the oxytalan fibres formed a fibre meshwork enveloping the surface of the root. From the cementum surface the majority of fibres increased in diameter as they arced apically toward the vessels in the ligament, particularly those adjacent to the alveolar wall. The meshwork had an essentially occluso-apical orientation. Major oxytalan fibres were interconnected by both large and fine intercommunicating branches. Oxytalan fibre tracts existed around vertical vessels. Crossover arrangements were present in the meshwork in the apical half of this region extending from the buccal and lingual sides of each root. Occasionally, canals containing

vessels penetrated the buccal and lingual plates. Histological staining did not reveal oxytalan fibres within the canals.

Region C

Oxytalan fibres curved into the periodontal ligament forming a dense intercommunicating meshwork surrounding each root apex. This oxytalan meshwork related the prominent vascular system at the apex to the adjacent cementum. Slightly apical to the crossover region the roots of all molars revealed major oxytalan fibres curving apically into the periodontal ligament at a characteristically less acute angle with the cementum. Direct connection between the mandibular nerve and the periodontal ligament of the mesial root of the first molar occurred in some animals but the oxytalan fibres were preferentially orientated to the vessels of the periodontal ligament and not to the nerve capsule. In the apical region canals were present in the bone providing communication of the molar periodontal ligament with marrow spaces, the mandibular nerve, and the incisor tooth. The oxytalan meshwork did not extend through these canals.

Region D

In the mid-interradicular region many of the larger oxytalan fibres had a vertical orientation between the cementum and the nearby network of vessels. Smaller communicating fibres contributed to an irregular meshwork arrangement straddling the apex of the interradicular crest. At the mesial and distal boundaries of the interradicular region the major oxytalan fibres became much longer and demonstrated the typical occluso-apical orientation seen in Region B.

On the buccal aspect the interradicular regions were associated with the nearby insertion of the masseter muscle in the case of the first molar, and the temporalis muscle with respect to the

second and third molars. From the buccal and lingual surfaces of the interradicular cementum some oxytalan fibres arising just below the cementoenamel junction extended horizontally while others curved toward the basement membrane of the gingivae. However, these oxytalan fibres did not pass across the buccal and lingual alveolar crests or mingle with the elastic fibres of the periosteum and muscle insertion. On the lingual side of this region no muscle attachment was present.

Region E

Mesial to the first molar the tendinous insertion of the masseter muscle extended across the alveolar crest or into the coronal portion of the periodontal ligament via a cleft in the alveolar wall. This cleft was frequently located to the buccal and lingual sides of the mid-region. In the gingival tissue slightly occlusal to the level of the epithelial attachment horizontal oxytalan fibres could be seen extending in a buccolingual direction. These fibres were part of the circular group which surrounded the crown of the tooth. Midway through this region of the periodontal ligament vertical fibres were observed which represented two different portions of the oxytalan fibre meshwork. The vertical fibres occlusal to the level of the cementoenamel junction were part of the fibre group which curved upward from the cementum toward the basement membrane of the free gingivae. The second and more prominent group of vertical fibres were derived from those fibres which extended apically from the cementum.

Tangential Sections: mesial and distal root surfaces

These sections revealed a similar arrangement to that observed in tangential sections on the buccal and lingual root surfaces. The oxytalan meshwork possessed a predominantly

occluso-apical orientation with a fine subsidiary inter-communicating meshwork. Vessels of the periodontal ligament were also vertically orientated and linked to each other by lateral communicating branches. Oxytalan fibres formed fibre tracts around the principal occluso-apically orientated groups of vessels. Crossover arrangements in the oxytalan fibre meshwork were evident opposite the region between the middle and apical thirds of the root.

HORIZONTAL SECTIONS

Characteristic regional patterns occurred in the coronal portion of the periodontal ligament corresponding with regions A, D and E. At the level of regions B and C additional features were observed in the pattern of the meshwork.

Regions A, D and E

Beneath the basement membrane of the gingival epithelium the oxytalan fibre meshwork could be seen circumscribing the cervical region of each crown. The fibres of this circular arrangement were derived from the cementum and the oxytalan fibre plexus at the basement membrane.

Oxytalan fibre arrangements circumscribing individual teeth united along the buccal and lingual sides to form a continuous band encircling the three molars. Across the interproximal regions the circular fibre groups of adjacent teeth intermingled with each other. Fibres contributing to the circular arrangement appeared to have a biased orientation toward the distal (Figure 2.17).

Region D was noted for the presence of oxytalan fibres passing buccolingually through the interradicular region and fanning out mesiodistally and occlusally to unite with the circular portion

of the meshwork on each side of the tooth (Figure 2.18). Since all first and second molars had two roots they were encircled with a figure of eight arrangement of the oxytalan system. Large vessels straddled the interradicular bone between the buccal and lingual aspects of the molars. As the third molar usually had a single root it was customarily associated with a circular oxytalan fibre meshwork in the cervical region without the figure of eight arrangement (Figure 2.19).

In the interproximal regions between the circular fibre portion of the meshwork and the crest of the interseptal bone the fine fibre components of the transseptal portion of the oxytalan meshwork could be seen. Individual fibres did not extend from tooth to tooth but contributed to the meshwork linking the adjacent teeth.

At the level of the interseptal crest short oxytalan fibres could be seen radiating outward from the periphery of the cementum. These fibres were the horizontal portion of the major fibres which extended out from the cementum into the periodontal ligament before curving apically toward the peripherally located vascular system. Adjacent to the cementum around its circumference could be detected the fine fibres of the oxytalan plexus investing and inserting into the cementum.

Oxytalan fibres exhibited two types of vascular association within the periodontal ligament. Some fibres were randomly associated with individual vessels without showing preferential attachment to any particular vascular type. The second arrangement revealed the presence of unique oxytalan-vascular structures. In these structures the fibres were associated not only with the walls of individual arteries, veins, and lymph vessels, but the oxytalan meshwork also surrounded the total vessel complex (Figure 2.20).

Horizontal sections in the cervical region revealed the tendinous insertion of the masseter muscle across the alveolar crest mesial to the first molar. On the buccal aspect the muscle inserted into the periosteum and above the level of the crestal margin to juxtapose the cervical region of the periodontal ligament and the oxytalan meshwork. Alongside the buccal of the mandibular second molar the temporalis muscle inserted into the periosteum, part of which was directly associated across the buccal alveolar crest with the cervical region of the periodontal ligament (Figure 2.21). Buccal and distal to the third molar the temporalis muscle was also related to the periodontal ligament across the alveolar crest (Figure 2.22). The elastic fibres of the masseter tendon came into close proximity with the oxytalan fibre meshwork as did the insertions of the temporalis muscle.

Throughout the horizontal sections numerous canals containing vessels and their accompanying oxytalan fibre shunts passed through the interseptal and interradicular bone. As many as four canals arranged mesiodistally were seen alongside each other near the interproximal crest of some animals. Occasionally, vascular canals also extended between the periodontal ligament and the buccal and lingual periosteum but they did not possess oxytalan staining fibres.

Region B

From the occlusal to the apical portion of this region, the horizontal sections revealed a similar distribution of the oxytalan meshwork around the roots. Oxytalan fibres seen in cross-section as dense concentrations of minute dots principally located between the middle portion of the periodontal ligament and the vessels adjacent to the alveolar wall. This distribution resulted in a dense concentration of the meshwork between the mid-portion of the periodontal

ligament and the vessels. Between the vessels encircling the roots the meshwork showed an accumulation of oxytalan fibres arranged occluso-apically. The oxytalan meshwork also provided lateral linkages from vessel to vessel. At the occlusal limit of this region principal vessels and their associated oxytalan fibre meshworks were situated slightly further from the alveolar bone than in the middle and apical portions where many vessels approximated the alveolar wall (Figure 2.23). The fibre meshwork did not extend between the vessels and the alveolar bone unless it was in a region where a major vessel penetrated the bone. Under these circumstances some fibres were observed to accompany the vessel into the superficial portion of the opening after which they could not be identified with conventional oxytalan staining methods.

The two types of oxytalan-vascular association were present throughout this region. Marrow spaces in the interseptal and interradicular bone linked adjacent teeth and the roots of individual teeth.

Region C

At the very apical region of the roots the fibres of the oxytalan meshwork radiated inward below the apical region to envelop the end of the root and enter the walls of the apical vessels. Canals were very plentiful in the apical region. These canals were observed linking the apices of the individual molars, as well as providing communication between the apical periodontal ligament of molars with the periodontal ligament of the adjacent incisor and the canal containing the mandibular nerve.

DISCUSSION

The consistency with which the present investigator could stain oxytalan fibres with aldehyde fuchsin in the mouse supports the view of Fullmer et al. (1974) that this transitory dye is particularly selective for oxytalan fibres after oxidation with monopersulphate. In conjunction with other histological techniques for collagen, reticulin, elastic, and nerve fibres, this morphological study clearly distinguished the oxytalan fibre as a unique structure within the periodontium (Fullmer 1958, 1967b; Fullmer and Lillie 1958; Fullmer et al. 1974). Despite the reported use of ten times the recommended concentration of basic fuchsin in Gomori's aldehyde fuchsin, Löe and Nuki (1964) reported haphazard results. Their staining difficulties could readily be attributed to unsatisfactory peracetic acid (Fullmer 1969), and hence the prolonged staining they employed with aldehyde fuchsin, or the use of deteriorated paraldehyde (Moment 1969; Fullmer et al. 1974). Inadequate maturation of the aldehyde fuchsin (Fullmer et al. 1974) undoubtedly contributed to the additional problems of background staining which Löe and Nuki failed to overcome. Neural and oxytalan staining elements in the mouse periodontal ligament are quite distinct (Goggins 1966).

Evidence that mouse oxytalan fibres were arranged as a three-dimensional meshwork system which extended uninterruptedly from the first to the third molar provided new knowledge of this periodontal fibre component. Apart from the publication by Sims (1973) no investigator has described specific regional patterns of oxytalan fibre distribution within the periodontium of the mouse molar. Other regional patterns described in this Chapter have been the subject of additional publications (Sims 1975, 1977b). Although Carmichael (1968)

considers the oxytalan pattern of mouse molars and incisors to be essentially the same, he neither defines regional patterns of distribution nor describes oxytalan fibre continuity between adjacent molars.

Certain features of the oxytalan fibre arrangement in animals and man are described by Fullmer (1958, 1959b, 1960b, 1960c, 1961, 1962, 1963, 1965b, 1966a, 1967b), Kohl and Zander (1962), Rannie (1963), Goggins (1966), Fullmer et al. (1974) and Sampson and Sims (1977). Simpson (1967) mentions isolated details of the oxytalan fibre arrangement in the ruptured human periodontal ligament. He describes main anchoring fibres and at least three other fibre types including interlacing groups, those which run with collagen bundles, and elementary fibrils near the cement. In the mouse these particular fibres would correspond with the principal fibres, the subsidiary interlacing fibre meshwork, and the cementum plexus. Simpson's examination of detached fragments of human periodontal ligament did not permit the oxytalan fibre system to be anatomically related to other periodontal structures. Contrary to Simpson's claim, it is not usually the interlacing fibres which outline the vascular spaces, but the principal fibres in both the mouse and man (Sims 1973, 1975, 1976).

Previous investigators have attributed specific lengths to so-called individual oxytalan fibres in different species (Fullmer 1958; Löe and Nuki 1964; Gawlik 1965; Carmichael 1968; Markens and Oudhof 1976). In the mouse periodontal ligament, long fibres and fibre tracts were readily discernible. However, their constant branching arrangement made it impractical to define the precise extremities of individual fibre components in a meshwork and so measure their lengths. Nevertheless, it is convenient to retain the term "fibre" for descriptive purposes.

Sims (1973) has refuted claims that oxytalan fibres are inserted into the alveolar bone (Fullmer 1958, 1961, 1966a; Rannie 1963; Goggins 1966; Boese 1969), that they arise from it (Fullmer 1967b), or that they unite the tooth to the bone at the apex (Fullmer 1967b). Fullmer et al. (1974) recently reassessed earlier findings and concluded that the oxytalan fibre is rarely inserted in bone. The impression of anchorage in bone probably was due to the angle of section through the vessels and their accompanying oxytalan fibres as they pierced the alveolar wall. Isolated oxytalan fibres were seen within the mouse alveolar process. These fibre inclusions in bone were considered to be remnants of the principal oxytalan fibres of the meshwork which had reconstituted in a new location as the teeth moved horizontally and occlusally during physiologic migration (Sims 1976). Oxytalan fibres were also observed in regions of hypercementosis (Goggins 1966) common around the root apices of 21 and 28 day old mice indicating that hypercementosis in these young animals occurred more rapidly than degradation of the principal oxytalan fibres.

In contrast to previous observations by Fullmer (1959b, 1961, 1967b) oxytalan fibres branched prior to their association with vessels. Furthermore, the delicate oxytalan fibre plexus ensheathing the root surface was embedded in the cementum. This last-mentioned finding differed from that of Goggins (1966) in the human periodontal ligament. No definite evidence was obtained which suggested differences in the arrangement of the oxytalan fibre meshwork on the mesial and distal sides of molar roots consistent with the distal tooth migration which occurs in mice. The distal bias of fibres contributing to the circular portion of the meshwork in the cervical region provided the only indication of a possible modification related

to distal drift.

Fullmer (1967b) states that the distribution of oxytalan fibres in the mouse periodontium approximates that of man. In human tissues the largest and most numerous fibres are reported in the transseptal region (Fullmer 1959b, 1962, 1966a; Fullmer et al. 1974) and in the middle and apical thirds of deciduous teeth (Goggins 1966). By contrast, maximum oxytalan fibre size in the mouse occurred within the formation that arched over the interproximal crest, in the occluso-apically orientated fibre group, and within the cementum below the cementoenamel junction. The most numerous fibres per unit area occurred in the apical and transseptal regions.

Continuity of the oxytalan fibre system across the trans-alveolar region is reported to differ in mouse, man, and other animals. In the mouse the oxytalan system formed a prominent structure across the transseptal region whereas in man (Fullmer 1961; Goggins 1966), and some animals (Edwards 1968, 1971; Boese 1969; Parker 1972), oxytalan fibre continuity is claimed to be absent. These differences provide an interesting contrast with the study of Sampson (1975) which showed that transseptal oxytalan fibre linkage occurs in Australian marsupials.

The predominantly occluso-apical orientation of the oxytalan meshwork corresponded with the main pattern of the principal vessels in the mouse periodontal ligament reported in this study, and also descriptions of the arrangement and orientation of the vascular system in the periodontal ligament of rat molars (Kindlova and Matena 1962; Kindlova 1967; Garfunkel and Sciaky 1971). Moreover, the oxytalan meshwork of the cervical plexus of the mouse molar showed a similar orientation to the arrangement of the vessels in the marginal periodontium of the Rhesus monkey (Kindlova 1965), rats (Kindlova 1968),

and certain features of the vascular system in man (Sims 1975, 1976).

Within the molar periodontal ligament multiple anastomoses existed between the various branches of the occluso-apically orientated vascular complex. This system of vascular intercommunication was ideally arranged to permit the rapid topographical differentiation of vascular flow according to the physiological requirements of masticatory function. Since the dense meshwork of oxytalan fibres anatomically linked the vascular complex to the cementum, the basement membrane of the gingival tissues and the approximal teeth, it is considered that the anastomosing vessel complex may respond to complex functional patterns of axial and non-axial tooth movement, and gingival distortion, via the oxytalan fibre system. The marked anatomical correlation between the distribution of the oxytalan meshwork and the vessels, together with the characteristic involvement of oxytalan fibres with the vessel walls, suggests that these structures interact to perform a specialized physiological role.

Modifications in the general arrangement and orientation of the oxytalan fibre meshwork near the junction of the middle and apical thirds of the root provided evidence of adaptation to the functional demands of a fulcrum region. Various tipping movements occurring in mouse molars during the chewing cycle, and as a result of other functional activities (Lewin 1970), could account for this modification in the oxytalan meshwork. Experimental studies by Parfitt (1967) have shown the presence of a functional rotation centre in human molar teeth. Moreover, Birn (1966) has suggested an association between the regional distribution of the blood supply in the gingival, middle and apical thirds of the molar periodontal ligament of man and an axis of tooth movement in the middle region of the root. The relationship of the fulcrum to the oxytalan system in

the mouse is diagrammatically represented in Figure 2.24 where the principal arrangements of the oxytalan fibre meshwork with its marked regional variations are derived from the present investigation (Sims 1973, 1975, 1977b).

From Figures 2.19 and 2.24 it can be seen that the unique orientation of the mouse oxytalan meshwork revealed in the static histologic image is ideally arranged to exhibit directional sensitivity to any applied stress. Under axial occlusal loads applied to the teeth and gingivae, the occluso-apically orientated oxytalan fibres would tend to relax as the related collagen bundles were placed under tension. When the occlusal forces diminished, the collagen bundles would relax and the oxytalan system come under tension as the tooth regained its occlusal level. Horizontal loads would cause the tooth crown and apex to move simultaneously in opposite directions. Resulting variations in lateral tension could be registered by the cervical and apical oxytalan meshworks. In view of the association of the oxytalan meshwork with the vascular walls (Fullmer 1958, 1959b; Carmichael 1968; Sims 1973, 1975, 1976; Fullmer et al. 1974), it is considered that tensional variations in the deformable oxytalan meshwork are registered in the walls of the vascular system. Furthermore, in evaluating the overall distribution of the oxytalan meshwork from a functional point of view, it is necessary to regard the transseptal region as part of the periodontal ligament (Melcher and Eastoe 1969).

Insertions of masticatory muscles in the mouse suggest that the effects of muscle action are transmitted to the periodontal ligament and its oxytalan fibre meshwork. Communicating canals throughout the alveolar bone of the mouse interconnected the teeth in a manner similar to that reported in the rat (Garfunkel and Sciaky

1971). Consequently, contraction of the muscles of mastication together with other functional stresses placed on the molar teeth and their periodontal structures can be expected to influence the incisor tooth, its periodontal ligament and the associated structures. Loads applied to the incisor crowns will similarly transmit physiological effects to the periodontal structures supporting the molar teeth as a result of jaw distortion (Noble 1973). The oxytalan-vascular system is considered to be an integral component of the complex co-ordination between the various structures of the mouse masticatory apparatus in which the whole mandible acts as a functional unit (Picton and Slater 1972). Anatomically, therefore, the mouse mandible with its oxytalan fibre meshwork demonstrates an additional basis for an interrelationship in function between the teeth, the periodontal ligament, bone, gingivae, muscles and nerves, to that recognized by Kawamurra (1974) as occurring in the human masticatory system.

SUMMARY

1. Oxytalan fibres in the mouse formed a three-dimensional meshwork extending between the dentinocemental junction and the vessels in the periodontal ligament.
2. The meshwork continued over the interseptal alveolar crest to form a fibre system that extended uninterruptedly from the first to the third molar.
3. Distinct regional patterns were present in the oxytalan fibre meshwork.
4. The regional meshwork patterns coincided with the vascular distribution and functional patterns of axial and non-axial tooth movement.

5. Oxytalan fibres exhibited characteristic associations with the walls of all vessels within the periodontal ligament.
6. Anatomical relationships suggested that tooth oscillation, gingival distortion, and muscle function could affect the vascular system via the oxytalan fibre meshwork.
7. Orientation of the oxytalan fibre meshwork generally contrasted with that of the principal collagen fibre bundles.
8. Fibres of the meshwork did not insert into alveolar bone.
9. Compared with the collagen fibre system of the periodontal ligament, the oxytalan fibre meshwork formed a highly differentiated and co-ordinated fibre system.
10. In the absence of any valid refutation, the extrapolation of functional concepts is considered to represent informed speculation at this stage of our knowledge and awaits further experimental investigation.

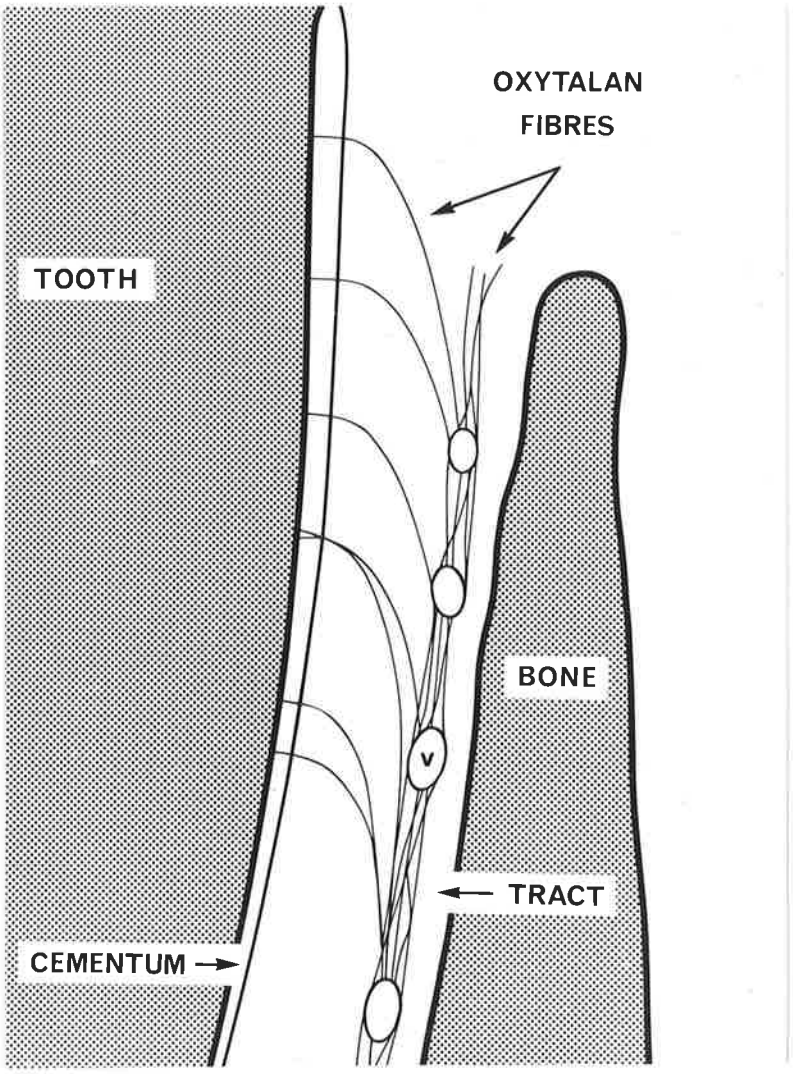

Figure 2.1 Schematic arrangement of the principal fibres of the oxytalan system in a mesiodistal section through the tooth. Principal oxytalan fibres extend from the dentinocemental junction to more apically located vessels. Periodontal vessels are also linked vertically by fibres or multiple groups of fibres forming tracts. C: cementum; OXT: oxytalan fibre tract; P: oxytalan fibre plexus; V: vessel.

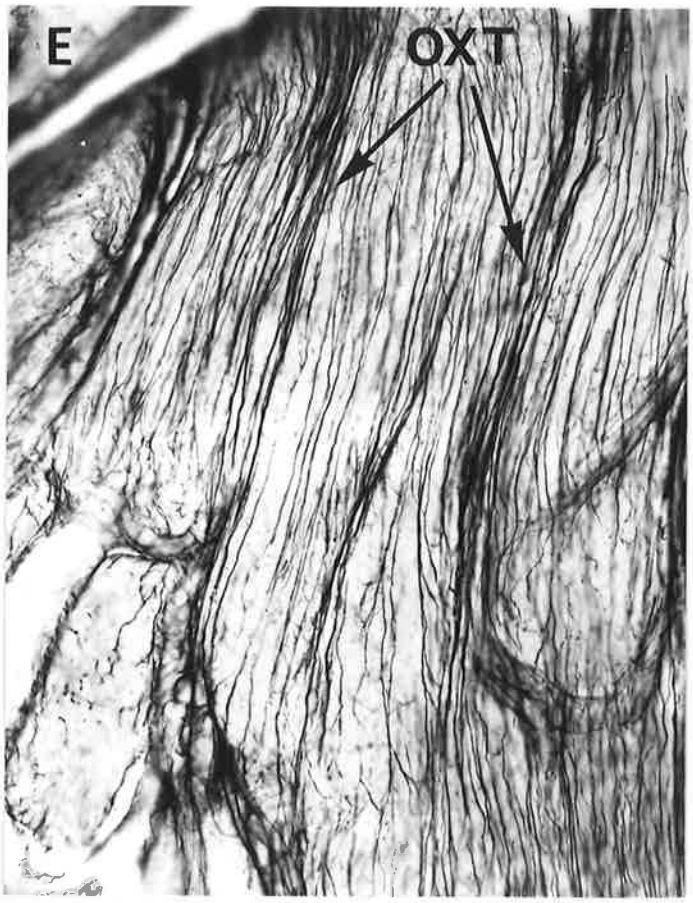

Figure 2.2 The predominant occluso-apical orientation of the oxytalan fibre meshwork and its association with the vascular system. Mesiodistal section through the periodontal ligament on the buccal aspect of a first molar. E: gingival epithelium;
OXT: oxytalan fibre tracts.
Oxone, aldehyde fuchsin, light green. X 250

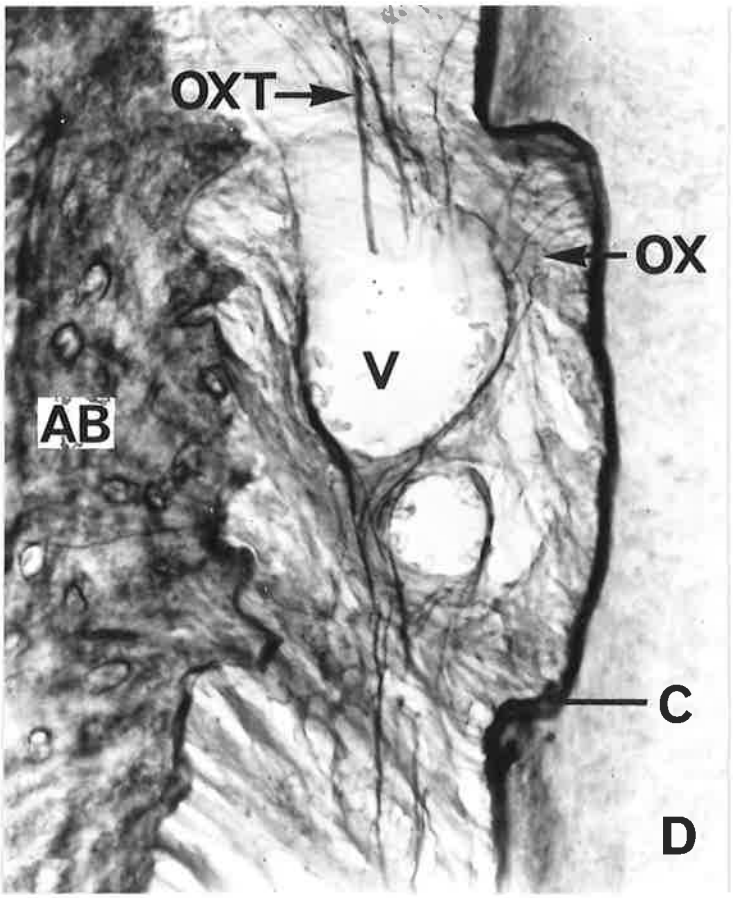

Figure 2.3 Dentine resorption and repair associated with the re-establishment of the oxytalan fibre system between the cementum and a vessel wall.
Mesiodistal section, distal of first molar.
AB: alveolar bone; C: cementum; D: dentine; OX: new oxytalan fibres; OXT: oxytalan fibre tract; V: vessel.
Oxone, aldehyde fuchsin, light green. X 400

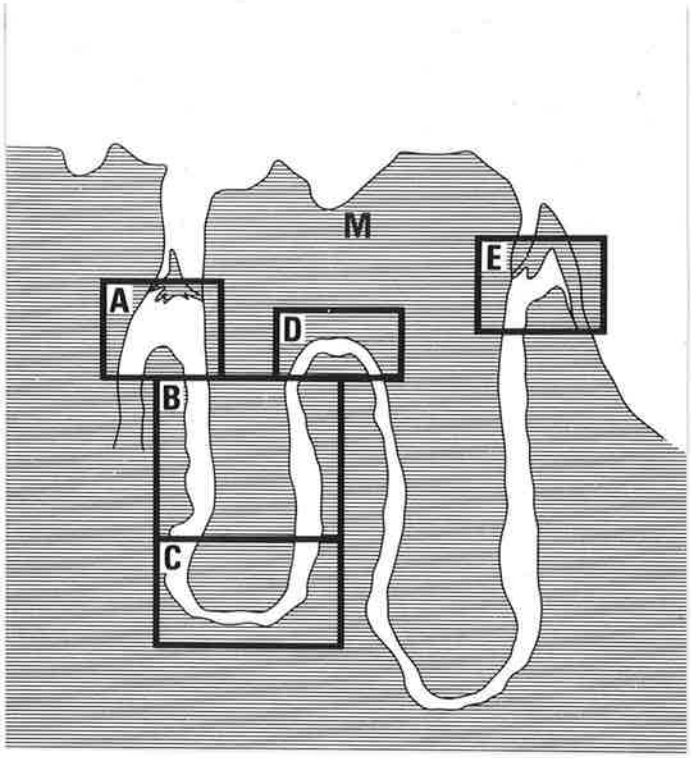

Figure 2.4 Five regions, A, B, C, D and E where the oxytalan fibres show different patterns of distribution in the mouse mandible. M: mandibular first molar.

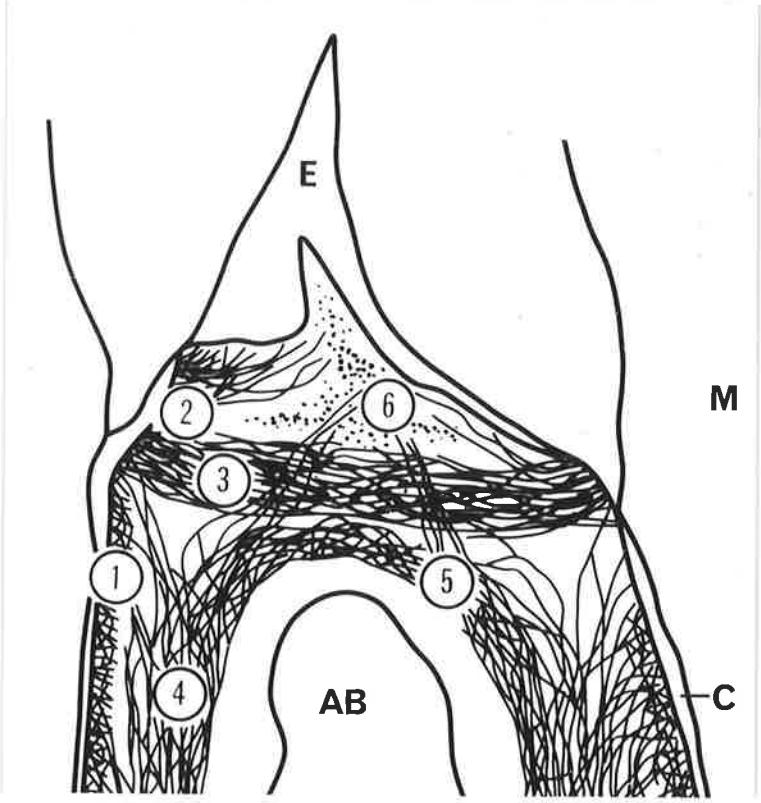

Figure 2.5 Diagrammatic representation of the six main oxytalan fibre groups in the interproximal site comprising Region A. Fibre diameters are not represented to scale. AB: alveolar bone; C: cementum; E: gingival epithelium; M: first molar.

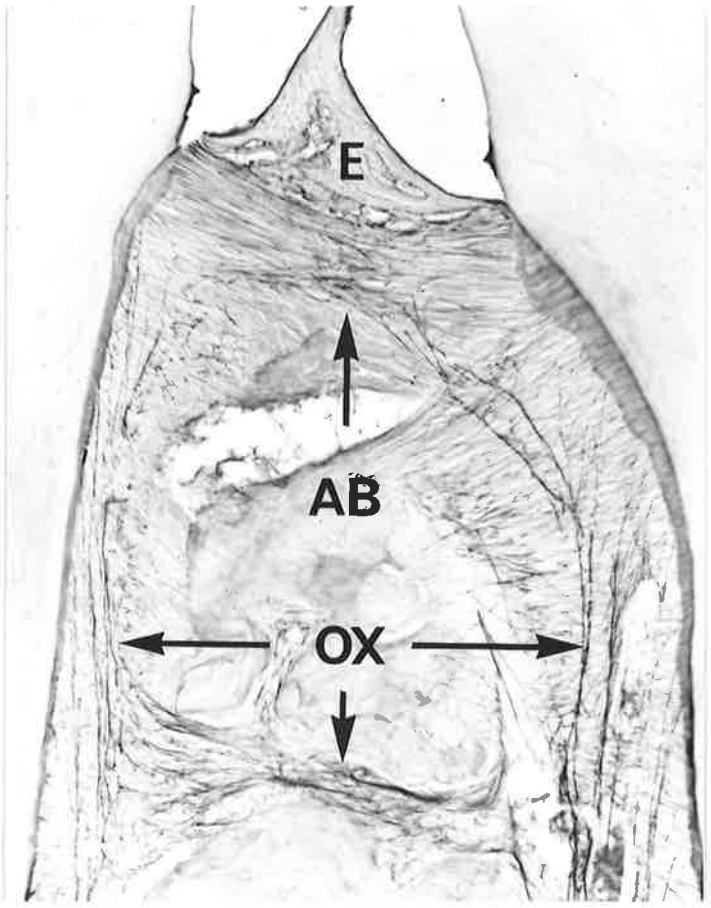

Figure 2.6 Oxytalan fibres accompany vessels traversing the interseptal bone between the first and second molars. The oxytalan meshwork also straddles the alveolar crest. AB: alveolar bone; E: epithelium of the interdental papilla; OX: oxytalan fibres. Oxone, aldehyde fuchsin, light green. X 250

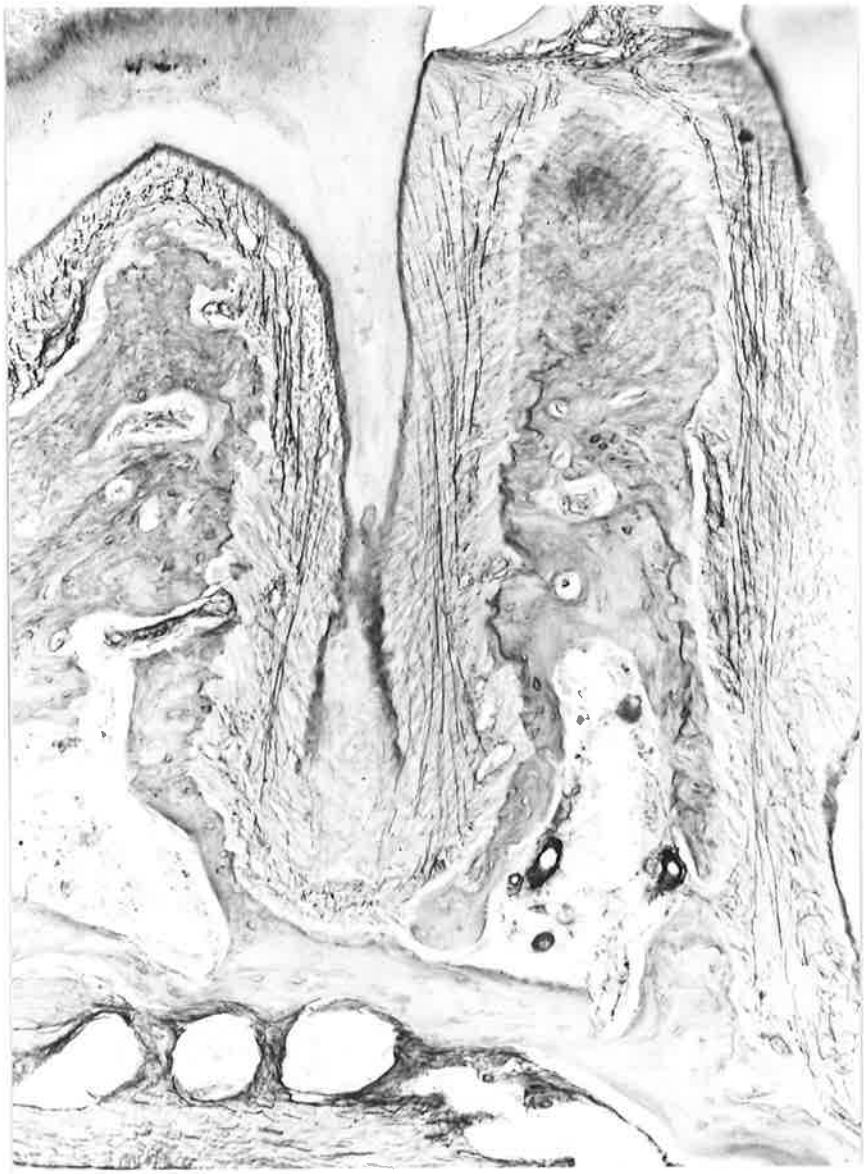

Figure 2.7 Mesiodistal section through the mesial root of a second molar illustrating the oxytalan fibre system in Regions A, B, C and D. The periodontal ligament and vessels of the lower incisor are seen at the bottom of the illustration. Oxone, aldehyde fuchsin, light green. X 120

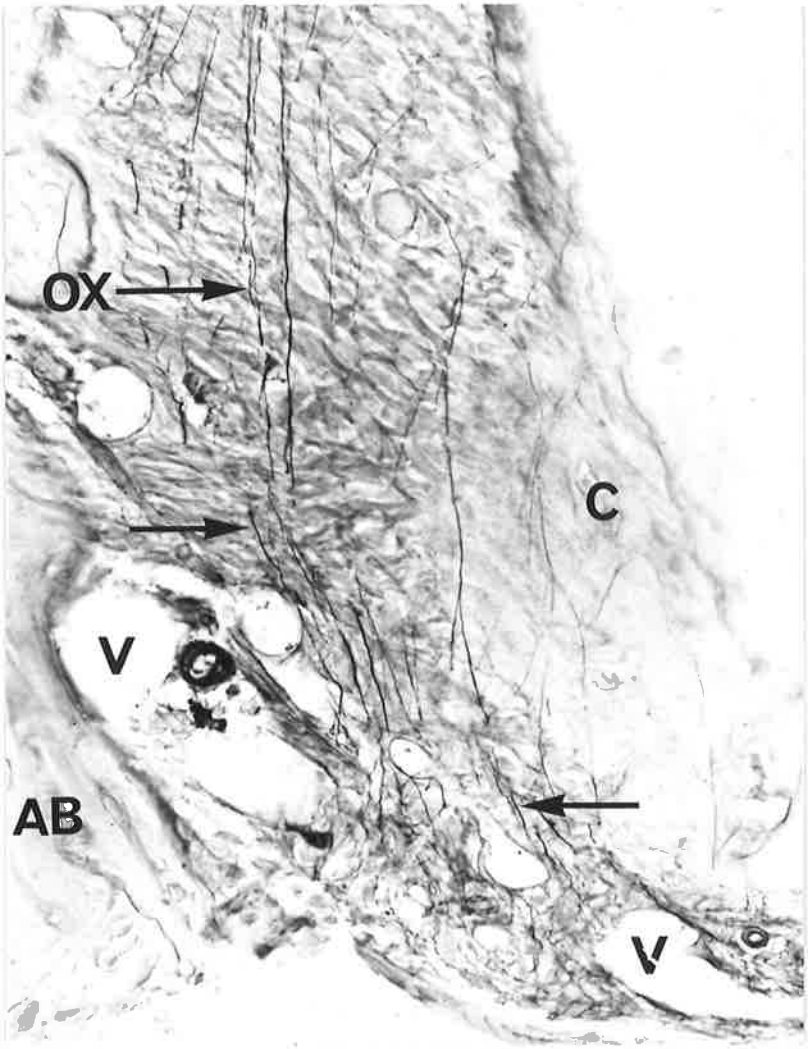

Figure 2.8 Arrows identify oxytalan fibres that enter the walls of vessels. Vessels are located below and lateral to tooth apex in Region C. AB: alveolar bone; C: cementum; OX: oxytalan fibres; PL: periodontal ligament; V: vessel. Oxone, aldehyde fuchsin, light green. X 250

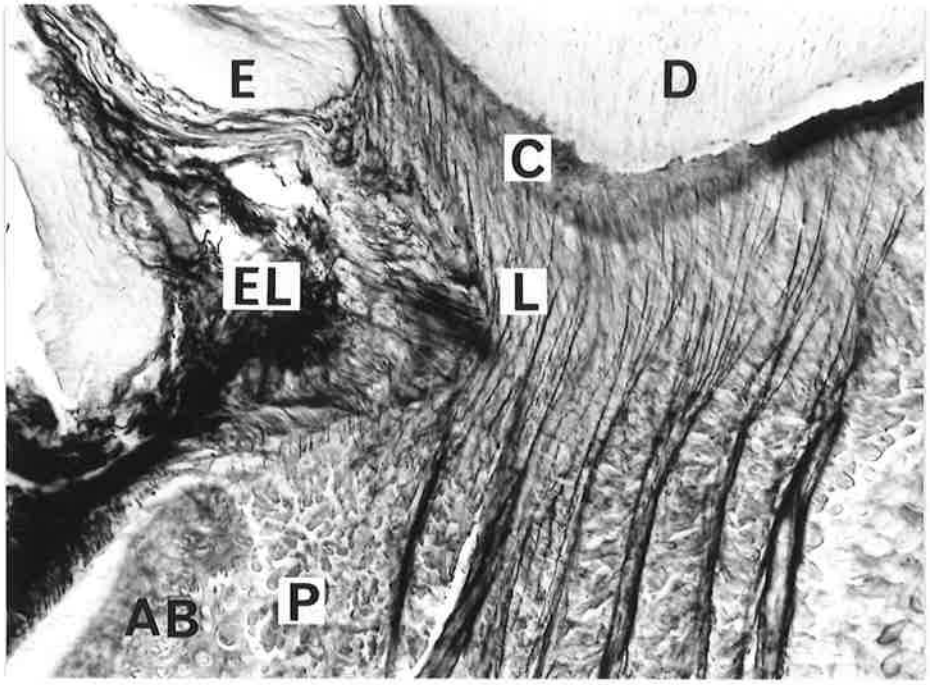

Figure 2.9 Tendinous extension of the masseter muscle into the periodontal ligament on the mesial of the first molar. The tendon contacts the oxytalan fibre meshwork extending from the dentinocemental junction into the walls of the vessels. Mouse on a lathyritic diet for seven days. AB: alveolar bone; C: cementum; D: dentine; E: gingival epithelium; EL: elastic fibres of tendon; P: periodontal ligament; L: tendon limit. Oxone, aldehyde fuchsin, light green. X 250

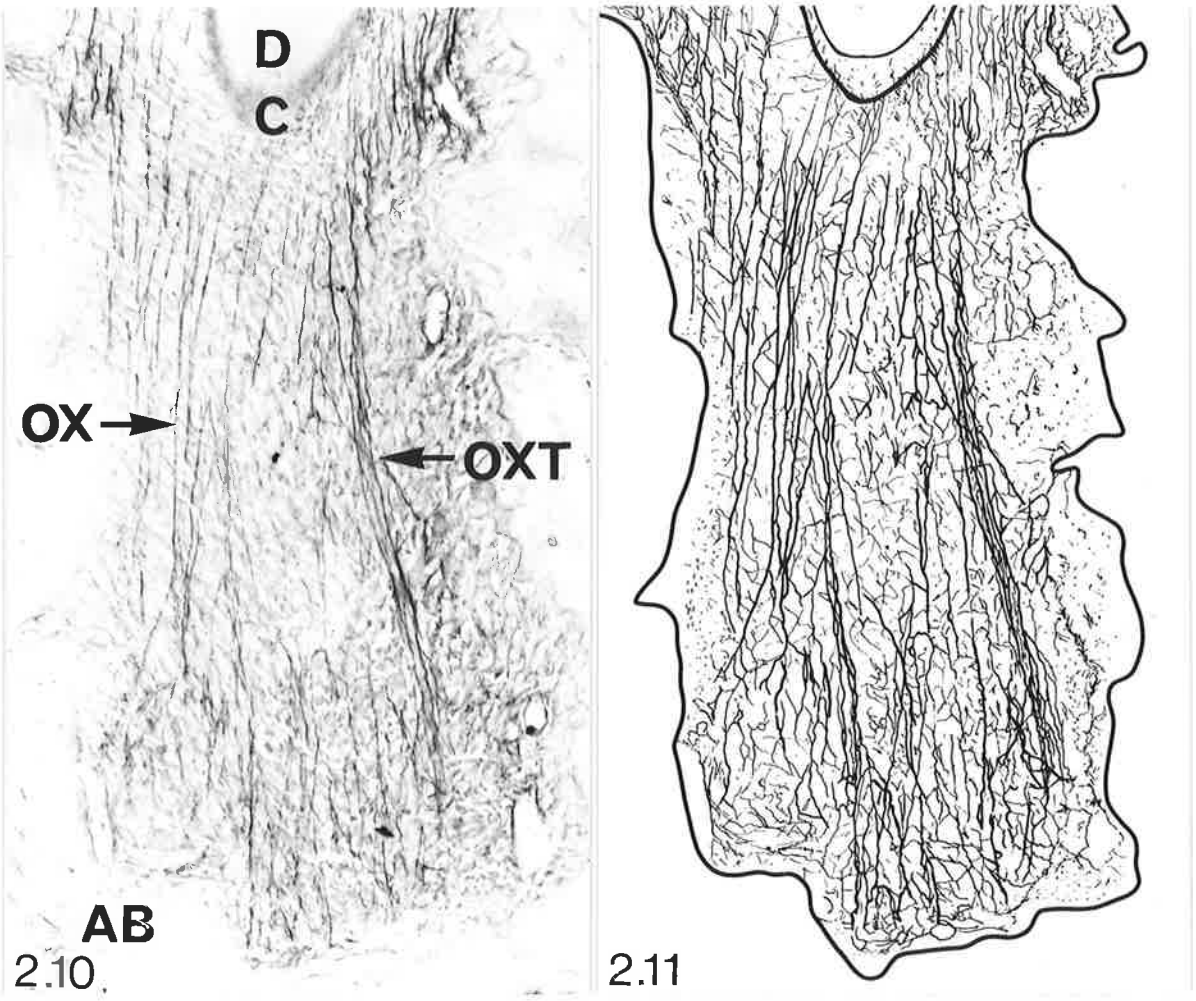

Figure 2.10 Composite photomicrograph of the oxytalan meshwork and tract formation revealed in a tangential section through the periodontal ligament alongside the buccal aspect of the root. AB: alveolar bone; C: cervical cementum; D: dentine; OX: oxytalan fibres; OXT: oxytalan tract.
Oxone, aldehyde fuchsin, light green. X 250

Figure 2.11 A tracing of the oxytalan fibre system seen in Figure 2.10, illustrating the density of the fibre meshwork. Obtained by focussing the microscope in stages through the full thickness of the 8 µm section. The diameters of the fibres are not drawn to scale.

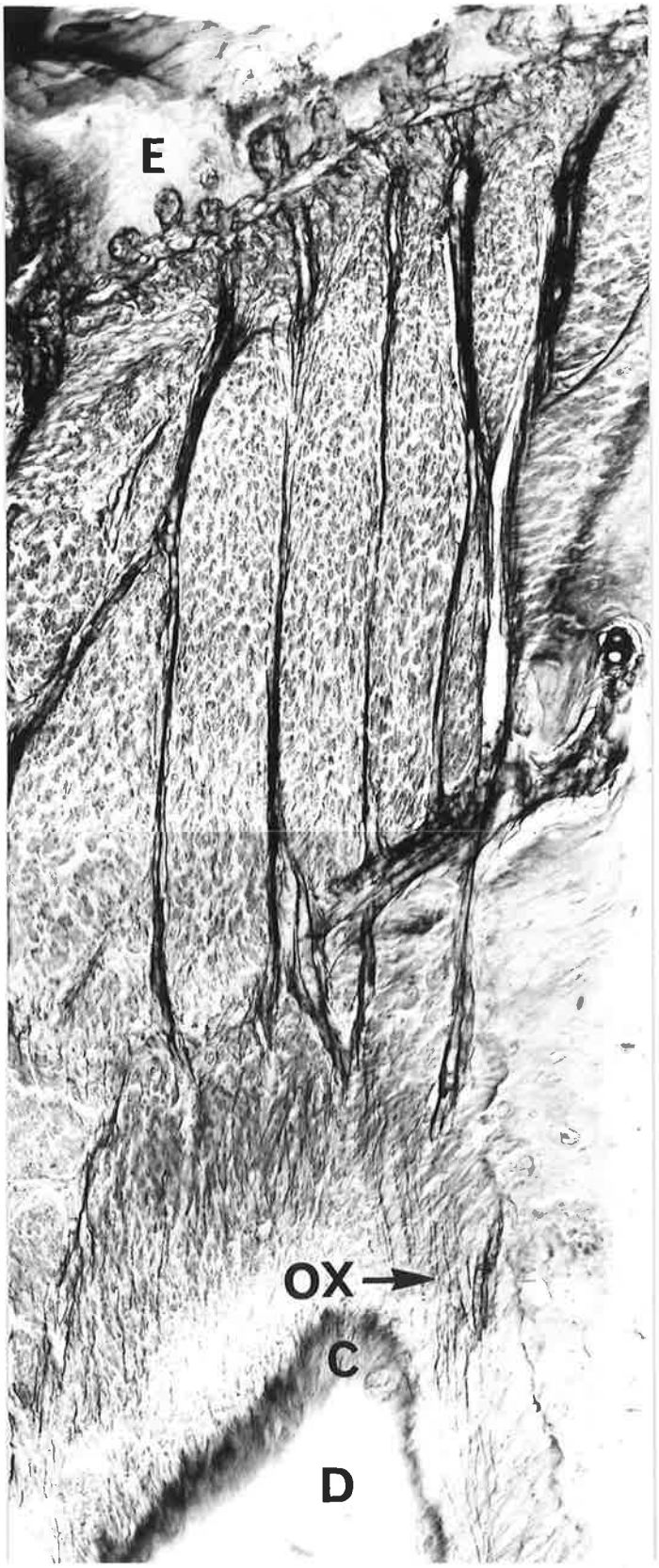

Figure 2.12 Vascular arrangement in the coronal half of the periodontal ligament. Lingual side opposite the mesial root of a mandibular first molar.
C: cementum; D: dentine; E: gingival epithelium; OX: oxytalan fibre meshwork.
Oxone, aldehyde fuchsin, light green. X 250

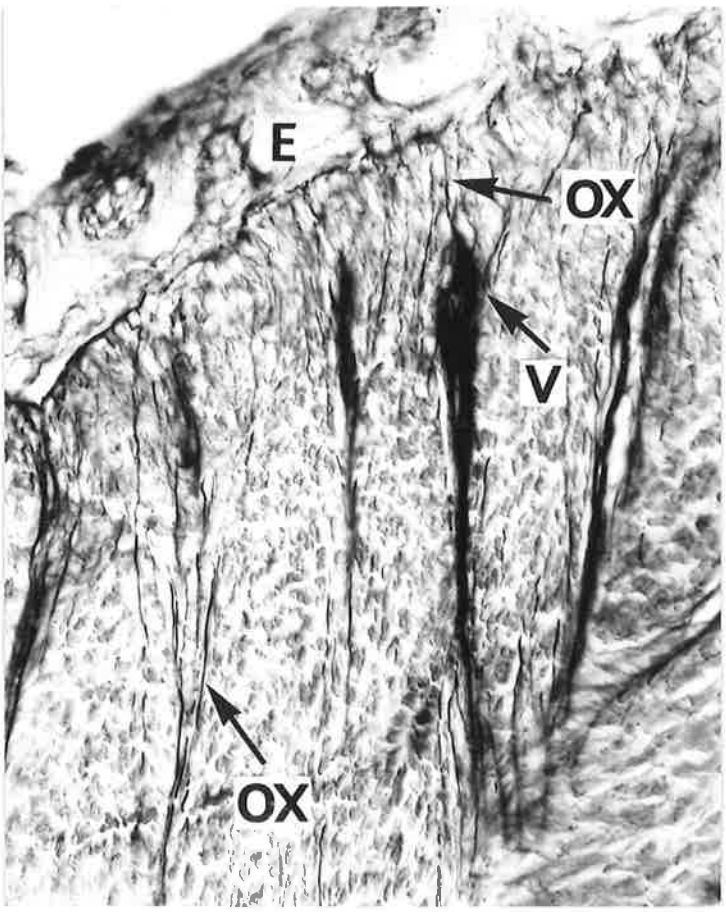

Figure 2.13 Oxytalan fibres extend from the subepithelium region into the walls of the occluso-apically orientated vessels. Adjacent section to Figure 2.12. E: gingival epithelium; OX: oxytalan fibres; V: vessel.
Oxone, aldehyde fuchsin, light green. X 395

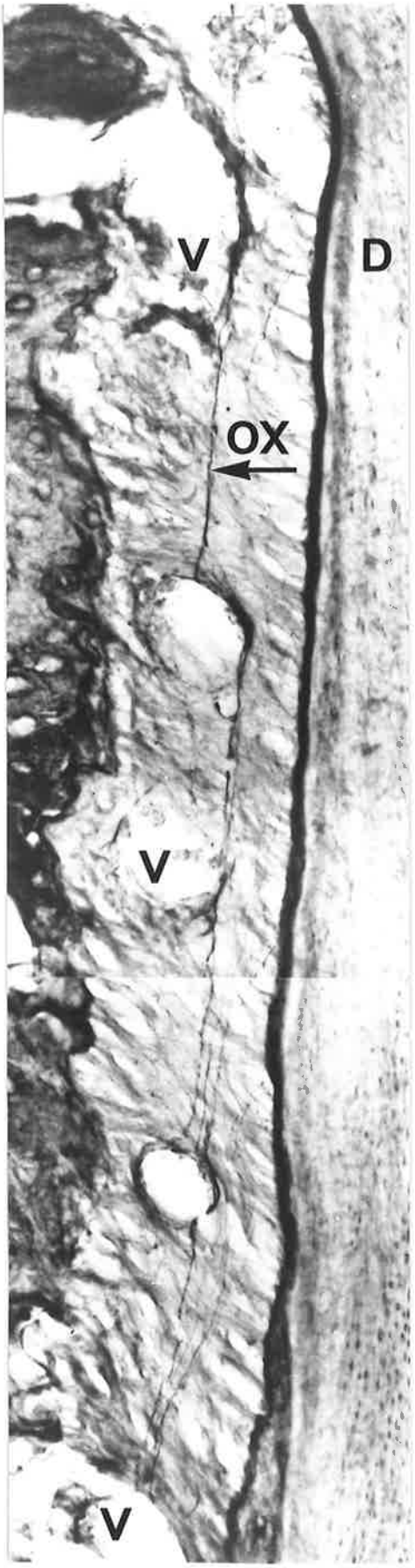

Figure 2.14

Occluso-apical linkage of communicating vessels by fibres of the oxytalan meshwork. Composite photomicrograph alongside the distal root of a first molar from the interradicular to the apical region.
AB: alveolar bone;
D: dentine; OX: oxytalan fibres; V: vessel.
Oxone, aldehyde fuchsin, light green. X 250

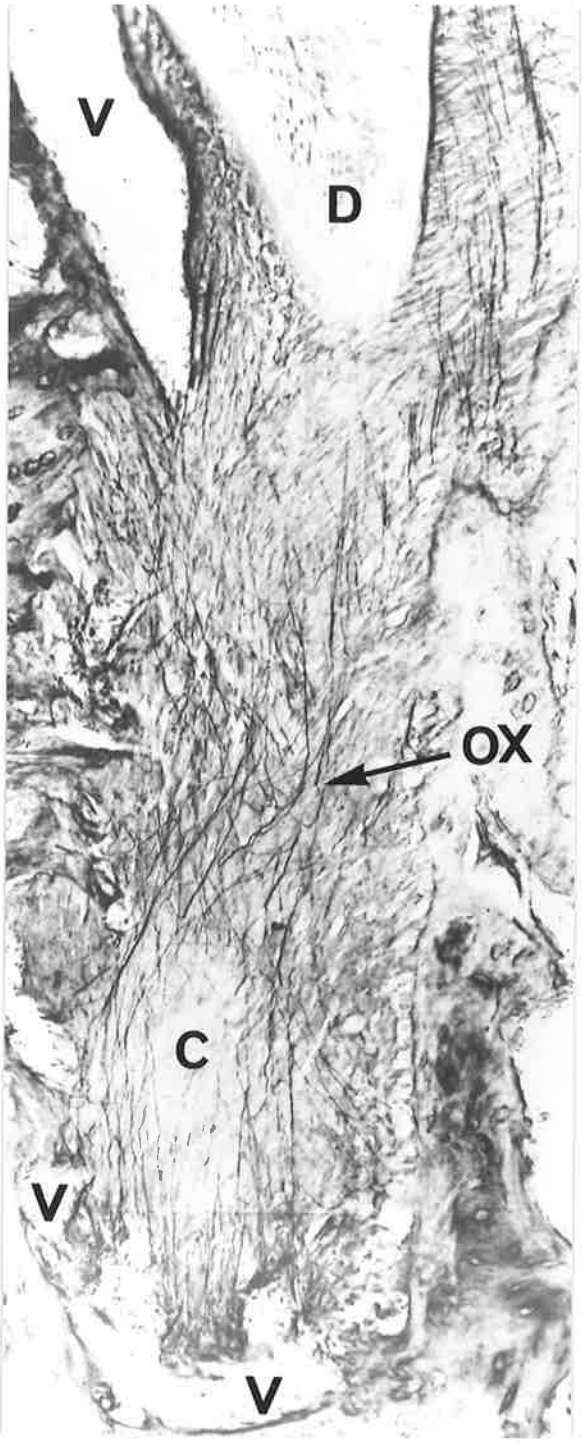

Figure 2.15 Crossover arrangement in the oxytalan fibre meshwork in the form of fibre loops at the junction of the middle and apical thirds of the mesial root of a second molar. Mesiodistal section.
C: cementum; D: dentine; OX: oxytalan fibre crossover; V: vessel.
Oxone, aldehyde fuchsin, light green. X 187

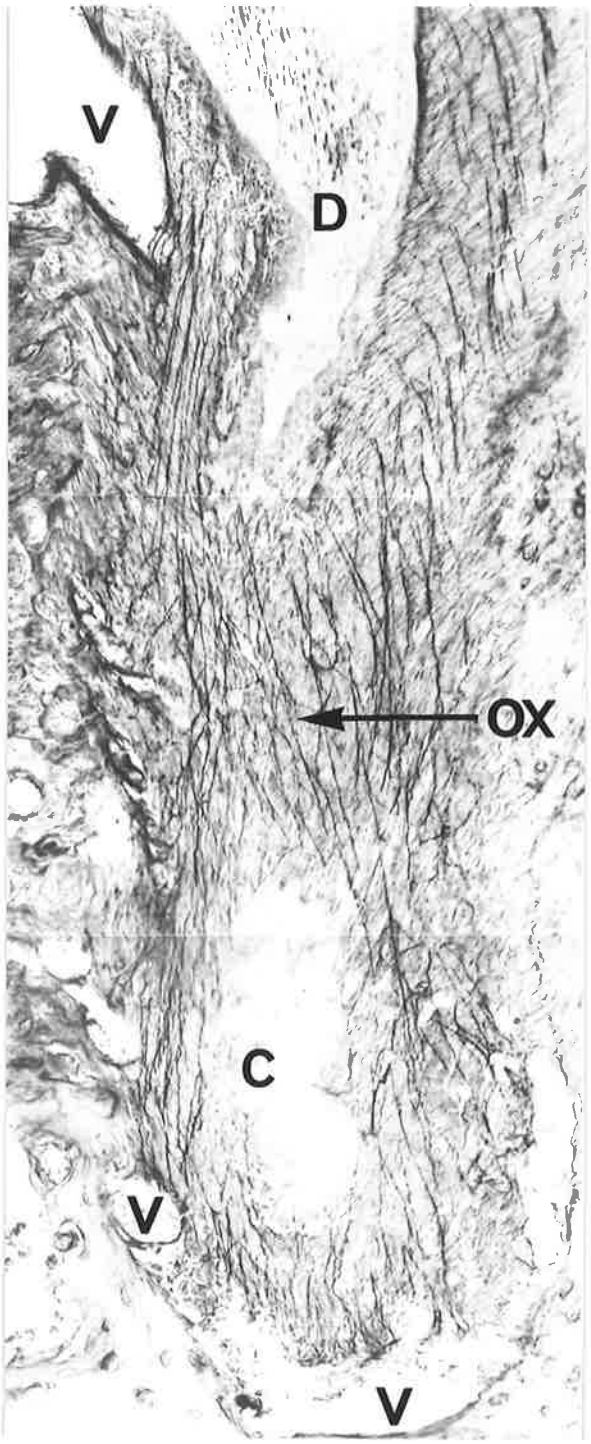

Figure 2.16 Major oxytalan fibres of the crossover formation also slope occluso-apically from the distal (left) side to the mesial (right) side of the root. Contiguous section to Figure 2.15. C: cementum; D: dentine; OX: oxytalan fibre crossover; V: vessel.
Monopersulphate, aldehyde fuchsin, light green.
X 187

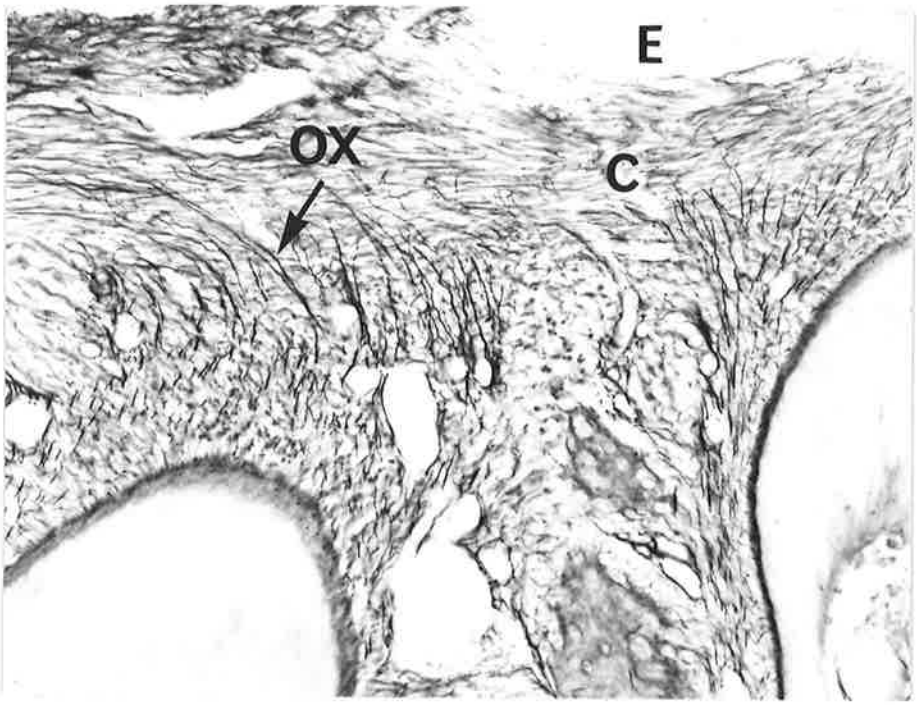

Figure 2.17 Oxytalan fibres on the buccal aspect of the second molar extend from the periodontal ligament to merge with the circular oxytalan fibre group. The fibres arising from the distal root have a distinct curvature to the distal. Oblique section.
C: circular fibres; E: gingival epithelium;
OX: oxytalan fibres with distal bias.
Oxone, aldehyde fuchsin, light green. X 240

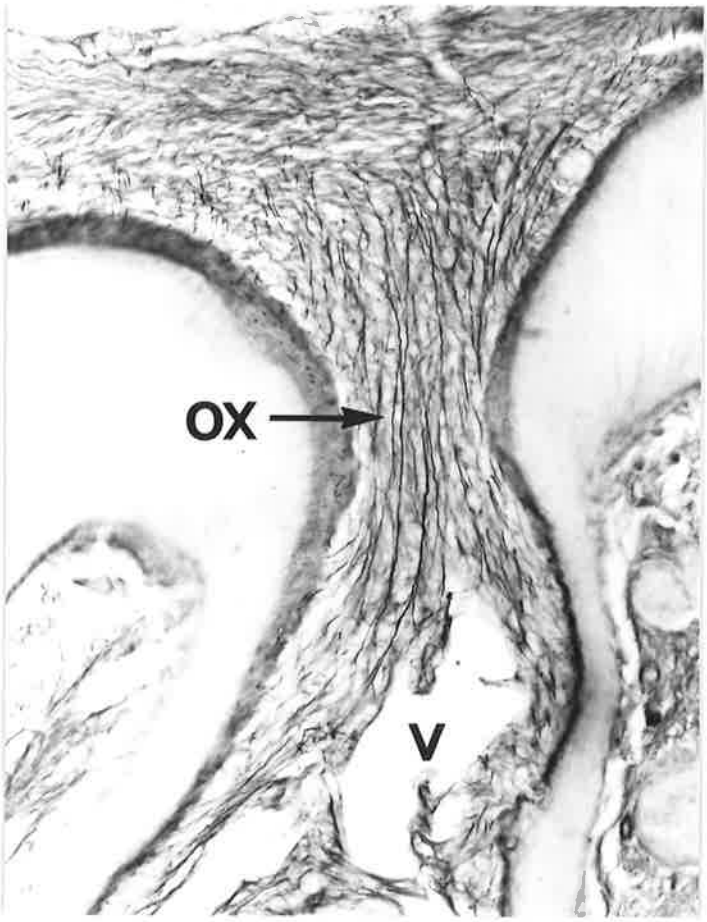

Figure 2.18 Oxytalan fibres passing through the interradicular region of a second molar. These fibres form part of the figure of eight arrangement around the cervical portion of the tooth. Horizontal section.
OX: oxytalan fibres; V: vessel.
Oxone, aldehyde fuchsin, light green. X 290

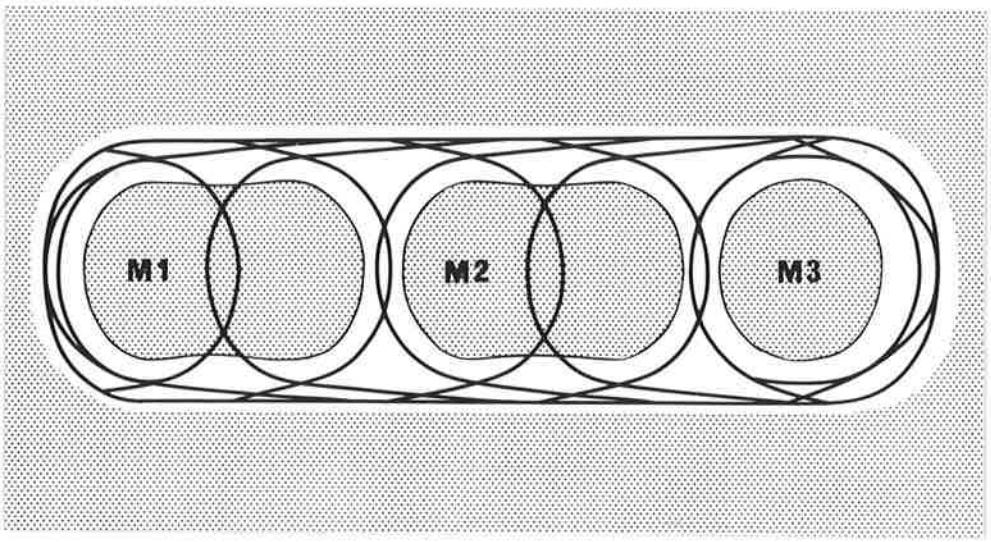

Figure 2.19 Diagrammatic representation of a horizontal section showing the oxytalan fibre system which encircles the cervical region of the mouse molars. M1, M2, M3: first, second and third molars, respectively.

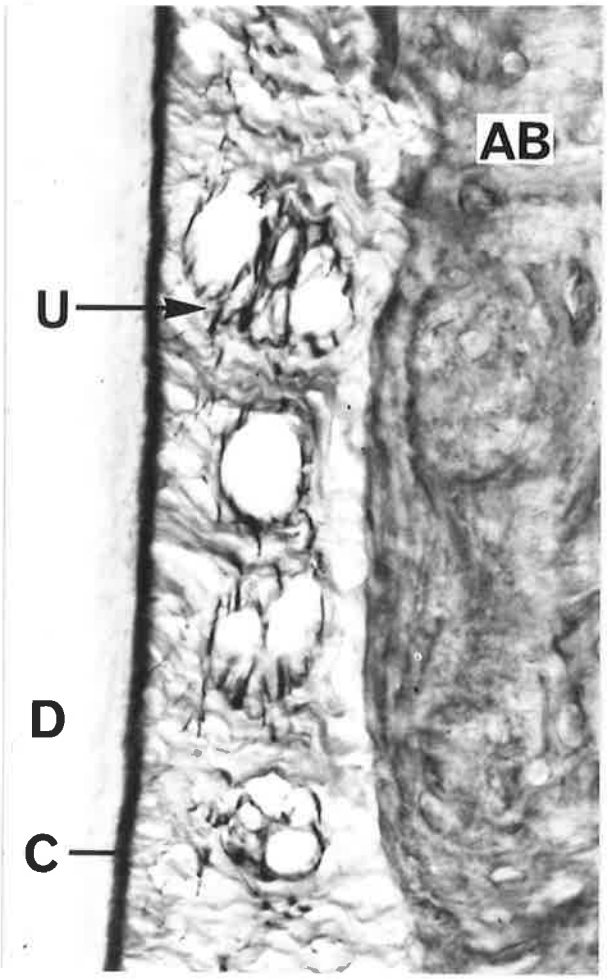

Figure 2.20 Mouse oxytalan-vascular units on the mesial side of a second molar. AB: alveolar bone; C: cementum; D: dentine; U: oxytalan-vascular unit. Oxone, aldehyde fuchsin, light green. X 545

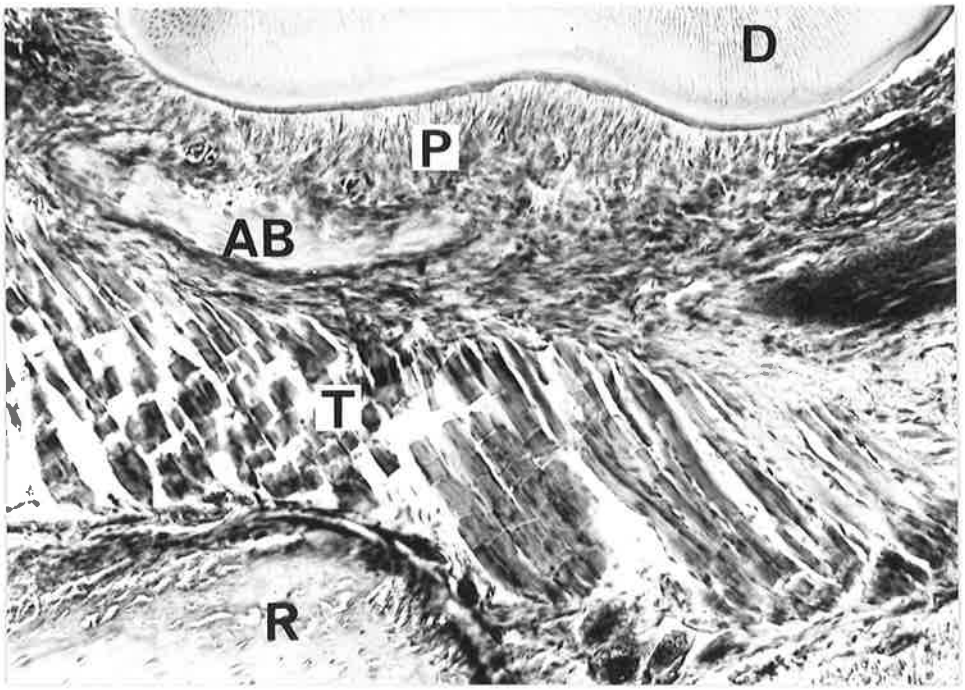

Figure 2.21 Temporalis muscle fibres in juxtaposition to the cervical portion of the periodontal ligament of the second molar. Horizontal section. AB: buccal alveolar crest; D: dentine of second molar; P: periodontal ligament; R: ramus; T: temporalis muscle.
Haematoxylin and eosin. X 180

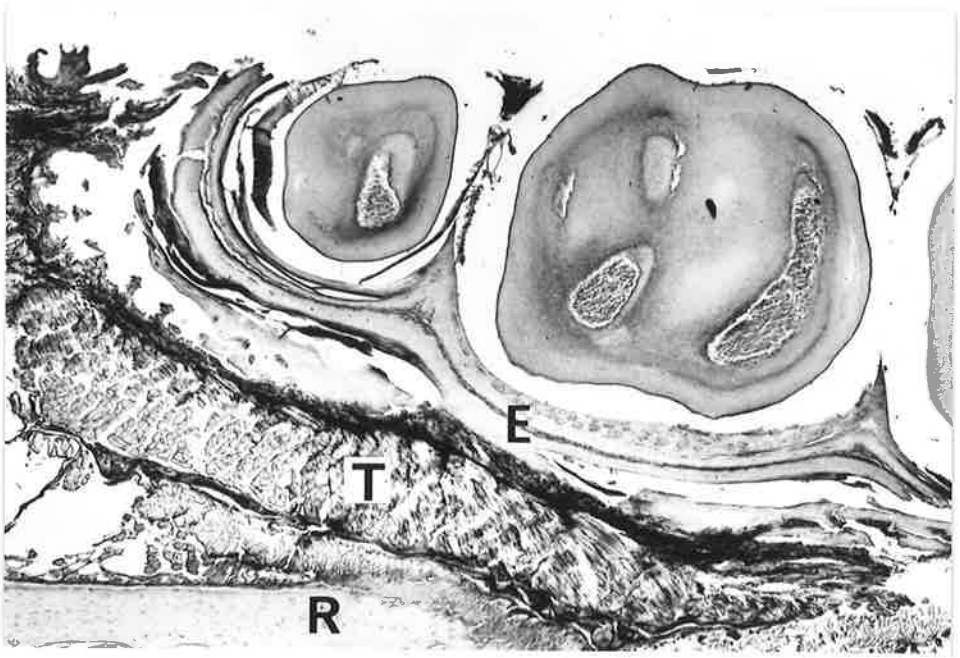

Figure 2.22 Temporalis muscle insertion in the second and third molar region. Horizontal section. E: gingival epithelium; R: ramus; T: temporalis muscle.
Haematoxylin and eosin. X 57

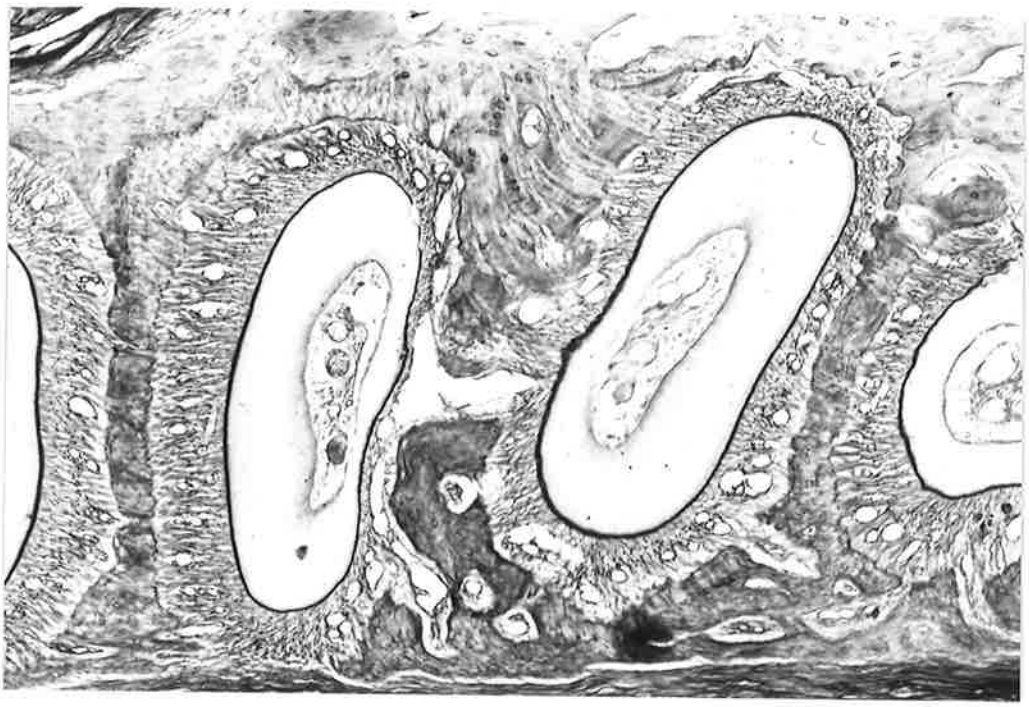

Figure 2.23 Vessels linked by the oxytalan fibre system encircle the two roots of the second molar. A large vessel extends across the interradicular bone. Horizontal section.
Oxone, aldehyde fuchsin, light green. X 100

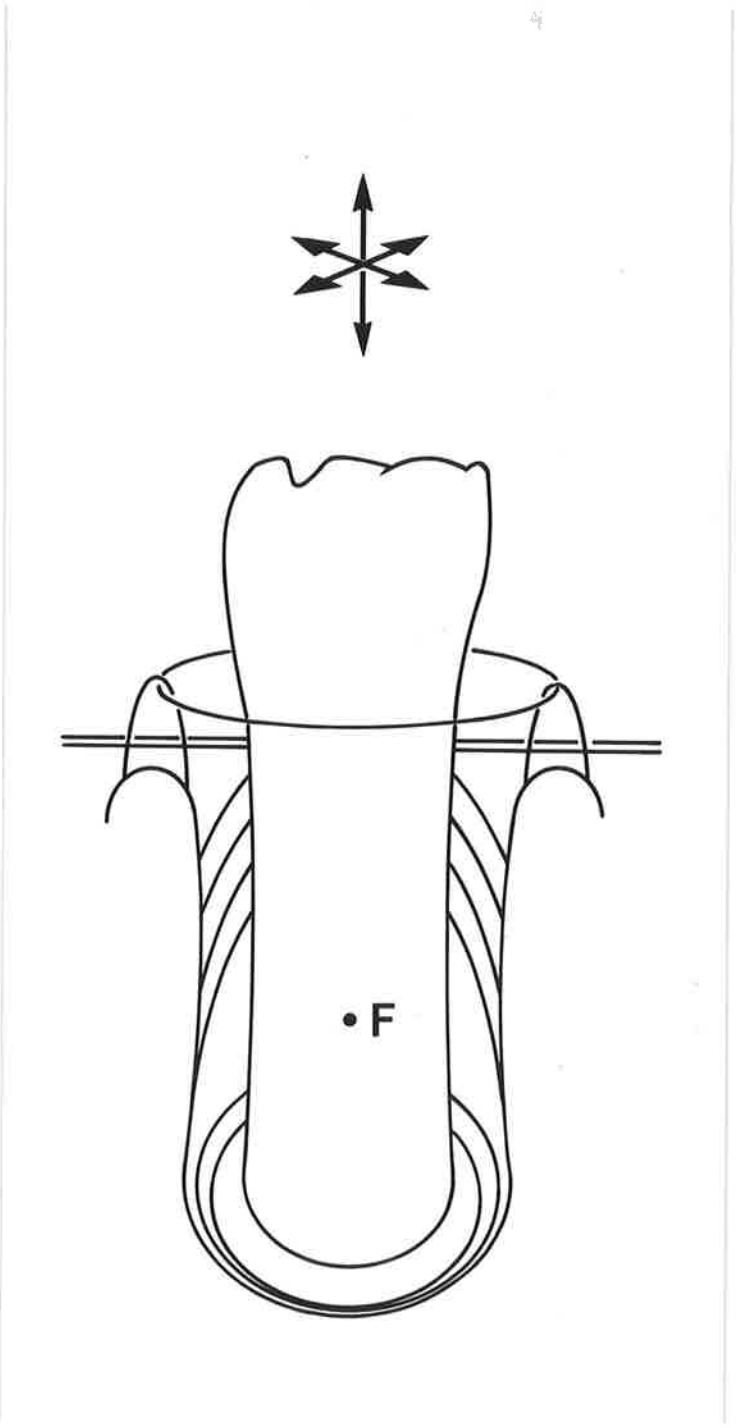

Figure 2.24 Diagram illustrating the principal arrangements of the oxytalan fibre meshwork and the fulcrum region in a mesiodistal section. The mandibular second molar of the mouse is depicted as a single rooted tooth. F: fulcrum region.

CHAPTER III

THE OXYTALAN FIBRE SYSTEM IN THE MANDIBULAR PERIODONTAL LIGAMENT OF THE LATHYRITIC MOUSE

Experimental osteolathyrism in laboratory animals is characterized by abnormalities in tissues with a high collagen content such as bones, blood vessels and dental structures (Selye 1957; Tanzer 1965). Young rats are particularly susceptible to the active lathyrus factor beta-aminopropionitrile and a variety of other lathyrogenic agents (Menzies and Mills 1957; Selye 1957; Tanzer 1965; Levene 1967). Although Lewis and Schulert (1949) were unable to demonstrate lathyrism in white mice, Dasler and Milliser (1957) and McCallum (1958, 1965) have shown that mice can also be made lathyritic when placed on a diet containing 50% sweet pea seed (*Lathyrus odoratus*), or by the administration of various compounds possessing lathyrogenic properties.

The elastic components of various tissues are also reported to be affected by lathyrogens (Menzies and Mills 1957; McCallum 1965). While the oxytalan fibre is considered to be a distinct type of connective tissue fibre (Fullmer 1958, 1960c; Fullmer and Lillie 1958; Sheetz et al. 1973), it is believed that oxytalan fibres are related to elastic fibres (Fullmer 1960c; Fullmer et al. 1974). Several investigators have described the effects of lathyrism on the periodontal ligament of rats without examining the oxytalan fibre (Gardner, Dasler and Weinmann 1958; Krikos, Morris, Hammond and McClure 1958; Gardner 1959; Krikos 1959; Sciaky and Ungar 1961; Sarnat and Sciaky 1965) although the presence of oxytalan staining structures has

been referred to by Gardner (1960) and Kennedy and Kennedy (1963).

The purpose of this investigation was to examine the effect of lathyrism upon the oxytalan fibre system, and test the hypothesis that oxytalan and collagen fibres are metabolically and structurally distinct.

MATERIALS AND METHODS

Mice were selected from related litters and randomly allocated to experimental and control groups. Males were used to obviate sex differences (McCallum 1965). All animals were 28 days old and had an average weight of 20 g. The experimental group was fed *ad libitum* a diet comprising equal parts of crushed sweet pea seeds (*Lathyrus odoratus*) and a proprietary brand of mouse cubes (Dasler and Milliser 1957; McCallum 1958) supplied by W. Charlick Ltd., Adelaide, Australia. Control animals were fed ground mouse cubes *ad libitum*. Both groups had free access to water and were housed at 22°C with constant relative humidity and regulated conditions to simulate night and day.

Pilot study

An examination was made of the progressive effect of lathyrism upon the periodontal ligament over a three month period using eight experimental and eight control mice. One experimental animal together with its control was sacrificed at weekly intervals during the first month. Thereafter, an experimental and control animal was sacrificed at fortnightly intervals during the next two months.

Principal investigation

On the basis of the histological findings in the pilot study

the investigation was repeated over a three month period using six experimental and six control animals in each of the eight groups. Animals were sacrificed at the end of 1, 2, 3, 4, 6, 8, 10 and 12 weeks. The mandibles of all 96 mice were examined.

Histological techniques

The heads were fixed in 10% neutral buffered formalin (Appendix I) for a minimum of 48 h after which the mandibles were removed, cleaned, and divided at the symphysis before being decalcified in formic acid. The mandibles were double embedded in celloidin and paraffin blocked (Appendix I). Serial sections were cut at 8 μm in the axial mesiodistal plane.

Tissues were examined with the light microscope after staining with a modified version of aldehyde fuchsin (Fullmer et al. 1974), orcein (Fullmer and Lillie 1958), PAS (Lillie 1965), 0.5% toluidine blue, and the Congo red and thioflavine T methods for amyloid (Pearse 1968). In addition, staining for acid mucopolysaccharides was carried out by the Steedman (1950) and Mowry (1963) methods cited in Pearse (1968). The ninhydrin technique for protein, haematoxylin and eosin, and the van Gieson stain were used according to Lillie (1965). Before staining with aldehyde fuchsin or orcein the majority of sections were preoxidized with Oxone (Rannie 1963).

Photomicrographs under Heine phase contrast and dark field illumination were taken on an Ortholux microscope (E. Leitz, Wetzlar, West Germany) using a dry field condenser.

Enzyme digestions

Enzyme tests were conducted on the oxytalan fibre system of incisor and molar teeth in the lathyritic and control mice of the

principal investigation to provide matching data with previous studies. Each test employed four serial experimental and control sections incubated under constant conditions in the following sequence:

Slide 1	enzyme	+ Oxone (60 min)	+ aldehyde fuchsin
Slide 2	buffer	+ Oxone (60 min)	+ aldehyde fuchsin
Slide 3	oxidation (60 min)	+ enzyme	+ aldehyde fuchsin
Slide 4	oxidation (60 min)	+ buffer	+ aldehyde fuchsin

Experimental and control tissues were exposed to digestion with the following enzymes: β-glucuronidase (Sigma, type B-1), 15 mg in 50 ml in 0.1M acetate buffer, pH 4.5, for 48 h at 37°C (Fullmer 1966a); elastase (Sigma, Type 111), 1:1000 5.0 mg in 50 ml in Tris buffer at pH 8.8 for 60 min at 37°C (Fullmer 1960b); pepsin (Sigma, P7012), 1:1000 50 mg in 50 ml 0.1N HC1 for 30 min at 37°C (Fullmer 1960b). In control experiments buffer solutions replaced the enzymes. The sections were stained with aldehyde fuchsin before or following oxidation with Oxone.

Three independent observers were used to evaluate the effects of digestion upon the stainability and appearance of oxytalan and elastic fibres, and the fibre-like lathyritic material.

FINDINGS

Mice on the lathyritic diet showed the rapid development of skeletal and dental defects although no paresis or spontaneous deaths occurred. A common sympton after two weeks was the impairment of the landing reflex. Scoliosis and kyphosis of the thoracic spine were usually evident after the second or third week although the animals remained active and ate well. Macroscopically, the excised, cleaned mandibles of the lathyritic mice were of normal shape but they were

slightly smaller and visually more translucent than those of the control animals. Both experimental and lathyritic animals demonstrated a relative increase in mandibular size with age. There was little definitive evidence of histological alterations to the incisor periodontal ligament of the lathyritic mice (Gardner et al. 1958; Gardner 1959). Because major pathological changes were produced in the periodontal structures surrounding the molars of the experimental mice, these changes formed the main subject of the present study.

Severe pathological changes were evident in the molar periodontal ligament of all experimental mice after seven days. The collagen fibres of the periodontal ligament were finer and revealed increasing disorientation, whereas the transseptal collagen group appeared unaltered and seemed to maintain their normal orientation throughout the experiment. Initially, a generalized vascular proliferation occurred with the appearance of numerous thin-walled vessels. Thereafter, the vascularity of the periodontal ligament progressively decreased.

Aldehyde fuchsin staining of oxidized periodontal tissues was significantly enhanced in all lathyritic animals from the first week. Oxytalan fibres in these mice required only three minutes for strong staining compared with the period of eight minutes used for control sections and the eight to twenty minutes recommended for routine staining (Fullmer and Lillie 1958; Fullmer et al. 1974). Aldehyde fuchsin applied to oxidized lathyritic tissues for eight minutes produced intense staining of many vessels, vessel groups, and their related oxytalan fibres, particularly those located in the coronal portion of the buccal periodontal ligament of the first and second molars (Figure 3.1).

Unoxidized control sections did not demonstrate oxytalan fibres or vessels after an eight minute stain with aldehyde fuchsin. Oxidized control sections treated with aldehyde fuchsin for the same time stained the oxytalan fibre meshwork but vessels failed to stain appreciably (Figure 3.2). The different staining reactions occurred consistently in all animals.

After one week of lathyrism, sections of periodontal ligament oxidized and stained with orcein revealed the presence of an additional oxytalan staining material with a fibre-like appearance which varied in width and length and frequently possessed a characteristic curving appearance. Distribution of the deeply staining fibre-like material was not uniform. This material had an orientation similar to that of the principal collagen bundles of the periodontal ligament, but differed markedly from the orientation and distribution of the established oxytalan system which remained clearly defined (Figures 3.3 and 3.4). The lathyritic material was generally confined to the mid-region of the periodontal ligament from the cervical crest to the apical region of the tooth. Where large vessels were present this material was very prominent. In some sections the fibre-like material extended across the periodontal ligament to approximate the cementum surface. Oxidation followed by aldehyde fuchsin treatment also revealed the fibre-like lathyritic material (Figures 3.5 and 3.6). Although this lathyritic material was occasionally demonstrated with aldehyde fuchsin in unoxidized sections, orcein staining did not reveal it without prior oxidation. In the molar periodontal ligament the lathyritic material gradually increased in amount from the first to the twelfth week and was consistently revealed with either orcein or aldehyde fuchsin (Figures 3.7 and 3.8).

During the first week, multiple lesions having an amorphous appearance developed around the roots of all molar teeth from the apical area to the region of the cemento-enamel junction. These lesions progressively increased in size until the eighth week when some extended almost across the width of the periodontal ligament. Amorphous lesions were rarely present at the apex of the interradicular regions nor were they observed directly above the interseptal crest where the transseptal collagen fibre bundles appeared to maintain their conventional orientation. Some oxytalan fibres traversed the amorphous lesions. These lesions also contained aldehyde fuchsin positive elements similar to the fibre-like lathyritic material (Figure 3.9). Where amorphous lesions enclosed portions of large vessels the vessel walls sometimes stained intensely with PAS (Figure 3.10). The oxytalan fibres associated with the blood vessels in these areas could be identified after oxidation and overstaining with aldehyde fuchsin.

Amorphous lesions lacked metachromasia with alcian blue and toluidine blue, gave negative results with the Steedman and Mowry techniques for acid mucopolysaccharides, stained lightly with PAS and failed to give positive reactions for amyloid or protein. Oxidation followed by aldehyde fuchsin stained the lesions a light pink colour while van Gieson stained them red as with collagen. Examination with Heine phase contrast illumination disclosed that the lesions were not homogeneous in structure but appeared to be traversed by collagen fibre bundles (Figures 3.11 and 3.12).

While substantial lathyritic changes were evident at the end of the first week some pathologic alterations in the molar periodontal ligament showed progressive development until the overall lathyritic condition appeared to become maximal after the eighth week.

Fibroblasts, for example, increased their cytoplasmic basophilia and became larger with more irregular and less distinct borders but these alterations showed signs of abatement after two months. During the investigation there was no significant change in the population of these morphologically altered cells. From the fifth and sixth weeks lathyritic mice showed marked bone resorption on both sides of the interradicular and interproximal bone, in contrast to the control sections where resorption occurred only on the mesial aspects of the bone (Figures 3.13 and 3.14).

Some tissue changes such as alveolar bone resorption and amorphous lesions showed signs of abatement after two months. However, when the experiment was concluded at the twelfth week, the lathyritic material in the molar periodontal ligament had not decreased although the major oxytalan fibres remained quite distinct (Figure 3.15).

Throughout the study the incisor periodontal ligament of the lathyritic mouse retained its typical arrangement of the oxytalan fibre system. The incisor oxytalan fibre system also stained more intensely (Figures 3.16 and 3.17) and vessels in the coronal portion demonstrated enhanced staining compared with those in control sections. Amorphous zones were not detected but some fibre-like lathyritic material was observed near the distal end of the root. The shape of the incisor was unaffected. Folds were not observed in the cementum.

The molar teeth showed significant intrusion at the end of eight weeks. As a consequence, the major oxytalan fibres exhibited a pronounced apical displacement in that part of their course between the cementum and the mid-region of the periodontal ligament. However, the displaced fibres maintained their tooth-vascular relationship as they passed through the periodontal ligament between the cementum and the blood vessels adjacent to the alveolar wall (Figure 3.18).

Enzymatic digestion disclosed no differences between the oxytalan fibres in the periodontal ligaments of the lathyritic and control mice in either the molar or incisor regions. Beta-glucuronidase treatment of unoxidised lathyritic and control sections, followed by aldehyde fuchsin staining, showed undigested molar and incisor oxytalan fibres as well as gingival elastic fibres. When lathyritic and control sections were preoxidized, the enzyme removed both molar and incisor oxytalan structures and only the undigested gingival elastic fibres could be stained. The lathyritic material responded to beta-glucuronidase in the same manner as the oxytalan structures.

Elastase digestion of unoxidized tissues failed to digest oxytalan but removed elastic fibres. When oxidation preceded the elastase-aldehyde-fuchsin sequence both oxytalan and elastic components were absent. Results were similar in lathyritic and control mice and also for the lathyritic substance. Pepsin treatment removed all oxytalan and elastic components from both unoxidized sections of lathyritic and control mice. These results were confirmed by a series of repeat digestions.

DISCUSSION

Dasler and Milliser (1957) did not observe skeletal changes in mice until after four weeks exposure to lathyrogenic agents and concluded that rats on comparable diets were more susceptible. McCallum (1965) reported that a 50% sweet pea seed diet produced only aortic lesions in mice, whereas rats suffered bone and aortic damage. Neither Dasler and Milliser (1957) nor McCallum (1958, 1965) examined the mouse periodontal ligament which this investigation proved to be a sensitive and reliable indicator of the onset of lathyrogenic changes. Although

the rat is considered to be particularly susceptible to lathyrism with the rapid onset of gross and microscopic lesions (Menzies and Mills 1957; Tanzer 1965) the early appearance and types of lathyritic changes in the mouse periodontal ligament were similar to those reported in the mandibular periodontal ligament of rat molars (Krikos et al. 1958; Krikos 1959, 1965; Gardner et al. 1958; Gardner 1959, 1960; Sciaky and Ungar 1961; Kennedy and Kennedy 1963; Sarnat and Sciaky 1965).

Contrary to the microscopic findings of previous investigations with lathyritic mice (Dasler and Milliser 1957; McCallum 1958, 1965), this study showed no spontaneous deaths of the experimental animals. There was a lack of the paresis reported by Selye (1957) and Menzies and Mills (1957) and, unlike the observations of Dasler and Milliser (1957) and Krikos et al. (1958), a reduction occurred in mandibular size. Furthermore, the mice showed no clear evidence of mandibular exostoses which occur in rats (Selye 1957; Gardner et al. 1958; Krikos et al. 1958; Kennedy and Kennedy 1963). Deformation of molar apices (Gardner et al. 1958; Kennedy and Kennedy 1963) and the abnormal incisor configuration recorded in rats (Gardner 1959; Sarnat and Sciaky 1965) did not occur in the mice. However, these differences could be attributed in part to variations in the degree of lathyrism induced during various studies and alterations in the rate of collagen synthesis in experimental species of different ages.

While the current study revealed some variation among individual animals, particularly in the observed time of onset and degree of spinal abnormalities, the overall pathological changes which occurred in the periodontal ligaments of all mice after seven days showed a progressive increase in severity during the next seven weeks.

A comparable peak response in the intensity of pathological changes in the rat periodontal ligament has been noted by Krikos et al. (1958). Such a response corresponds with the pattern of cellular stimulus and degeneration in the lathyritic rat aorta described by Ham (1962).

Recently, the author has completed a further study which demonstrated the onset of lathyritic changes in the mouse even earlier than previously reported (Sims 1977a). After three and four days on a 50% lathyritic diet the molar periodontal ligament revealed obvious histological changes.

The altered resorptive pattern of interradicular and interseptal bone associated with the reversal of distal molar drift in normal mice to mesial molar drift in lathyritic mice also occurs in rats (Gardner et al. 1958; Gardner 1959; Sarnat and Sciaky 1965). The reason for this change is not known. The resorptive bone changes may have been a reflection of the systemic effects of the lathyrogen or may have resulted from intrusion of the teeth due to the impaired tensile strength of lathyritic collagen (Levene and Gross 1959) which reduces functional support from the periodontal ligament (Sciaky and Ungar 1961; Sarnat and Sciaky 1965). Moss and Picton (1974) believe that approximal migration of mandibular cheek teeth is due to contraction of the intact transseptal fibre system. Morphologically the transseptal fibres of lathyritic mice appeared to be the least disorganized collagen fibres in the periodontal ligament. Transseptal fibres are also reported to be unaltered in lathyritic rats (Krikos et al. 1958). Nevertheless, if collagen contraction *per se* is responsible for tooth migration, the cessation of distal molar drift in lathyritic mice and rats might also be attributed to lathyritic derangement of the other collagen fibre bundles which constitute the periodontal ligament.

In the past, investigations of lathyritic changes upon the circulatory system have been concerned predominantly with large vessels such as the aorta in mice (Dasler and Milliser 1957; McCallum 1958, 1965) and rats (Menzies and Mills 1957; Ham 1962). In the present study evidence of the rapid onset and maintenance of lathyritic changes in the walls of arterial microvascular structures of the mouse periodontal ligament provided support for the concept of angiolathyrism proposed by Gerzeli and Cucchi (1975). These authors state that lathyrism primarily alters the structural and dynamic properties of the myocardial capillary wall. Therefore, they propose that experimental angiolathyrism should be considered as a separate condition to osteolathyrism and neurolathyrism. McCallum (1965) states that only elastic vessels are affected by lathyrism. In view of the similarities which exist between elastic and oxytalan fibres (Fullmer et al. 1974) the staining changes observed in many of the vessels in the periodontal ligament extend Gerzeli and Cucchi's concept to include lathyritic involvement of peripheral vessels in the mouse periodontal ligament. Moreover, the increased intensity of oxytalan fibre and vascular staining suggested that the fibres and vessels in both molars and incisors were altered by the lathyrogenic toxin in some unknown manner. The inconsistent staining of arterial-like vessels with the Schiff stain could not be explained. It did not appear to be related to the age of the animals at a stage of normally rapid growth (Alper, Prior and Ruegamer 1968). Individual animal susceptibility or the influence of dietary variations upon the vessels and their subsequent Schiff reations was not investigated.

Histological dyes provided no positive information as to the nature of the fibre-like lathyritic material in the periodontal

ligament because aldehyde fuchsin is known to stain altered collagen (Fullmer and Lillie 1957) and also reveals elastic as well as oxytalan fibres (Fullmer 1958; Fullmer and Lillie 1958). While orcein also stained the fibre-like material both dyes are said to have differing modes of action (Mander et al. 1968; Lillie 1969). Tanzer (1965) states that the exact physical state of lathyritic collagen *in vivo* has been the subject of controversy and the weight of evidence indicates that lathyritic collagen is organised into fibrillar and fibrous tissue structures. The frequent fibre-like appearance of the lathyritic material in the mouse periodontal ligament orientated similarly to the major collagen bundles suggested that this material might be a form of lathyritic collagen. Collagen synthesis is not inhibited in lathyrism (Levene and Gross 1959) although some investigators consider that lathyritic collagen originates from new and old collagen whereas others believe it is derived only from newly synthesized collagen (Tanzer 1965; Levene 1967). The periodontal ligament in the mouse and other animals is distinguished by its rapid rate of collagen turnover (Carneiro and Fava de Moraes 1965; Melcher and Eastoe 1969; Rippin 1976). However, regional variations in collagen turnover reported to occur in radioautographic studies of the periodontal ligament (Carneiro and Fava de Moraes 1965; Melcher and Eastoe 1969; Rippin 1976) do not coincide with the distribution of the mouse lathyritic material. As far as the present investigation is concerned the fibre-like lathyritic material could only be distinguished from oxytalan fibres on a morphological basis, thus providing evidence of the lack of specificity of the aldehyde fuchsin stain. The development of a fibre-like material in lathyrism may be analogous to the appearance of abnormal fibres in other diseased states (Gillman, Penn, Bronks and Roux 1955) including scleroderma which affects the periodontal ligament (Fullmer

and Witte 1962).

The presence of amorphous lesions in mice is also a constant finding in the periodontal ligament of rat molars. In rats these lesions are described in various ways such as homogeneous eosin-staining material resembling hyaline and osteoid (Gardner et al. 1958), amorphous eosinophilic material (Krikos, Beltran and Cohen 1965) hyaline lakes and lathyritic bodies (Gardner 1959), altered collagen (Gardner 1960), lakes of intercellular material (Kennedy and Kennedy 1963) and homogeneous hyaline-like masses (Sarnat and Sciaky 1965). As with Gardner's (1960) study of rats a variety of staining methods failed to indicate the nature of the amorphous lesions in the mouse although the lesions gave a positive reaction for collagen with van Gieson. Phase contrast illumination confirmed that the amorphous lesions contained a fibrillar structure similar to collagen and which Krikos (1959) demonstrated with silver stain. In the present study and that of Gardner (1960) occasional oxytalan fibres and a considerable amount of lathyritic material were found to be present in the amorphous lesions of mice. However, in rats a distinction has not been made between oxytalan fibres and lathyritic material (Gardner 1960) or the inclusion of fibre-like lathyritic material in the amorphous lesions is not mentioned (Kennedy and Kennedy 1963).

According to Krikos et al. (1958, 1965) and Kennedy and Kennedy (1963) mechanical stress plays an important role in the manifestation of morphologic changes in the periodontal ligament of lathyritic rats. Unlike rats, the periodontal ligament of all three mandibular molars in mice showed the presence of large amorphous areas after seven days of lathyrism. This consistent pathologic change was independent of individual molar eruption time and the subsequent

period in occlusion. These findings suggested that the duration of exposure to a toxic lathyrogen must also be considered as a factor in producing gross pathologic changes. Therefore, the lesions of lathyrism may be due to the dual influence of masticatory forces and the lathyritic agent as suggested by Gardner (1959) and Sarnat and Sciaky (1965). If masticatory function is so significant it remains to be determined why lathyritic lesions are so pronounced in molars compared with incisors.

Fullmer (1958, 1967b) and Fullmer and Lillie (1958) have reported the use of enzyme digestion procedures to provide additional information on the nature of the oxytalan fibre. Fullmer (1960b, 1967b) and Fullmer et al. (1974) regard β-glucuronidase as being a most informative enzyme because of its definitive effects on the oxytalan fibre. There are certain qualifications to be considered in the use of these enzymes including the variation in specificity from batch to batch particularly with elastase (Fullmer and Lillie 1958) and β-glucuronidase (Fullmer et al. 1974), and the need to avoid certain fixatives (Fullmer and Lillie 1958; Fullmer 1967b) such as chromate.

Enzyme digestion tests demonstrated that incisor and molar oxytalan structures in lathyritic tissues responded like their oxytalan counterparts in normal periodontal ligaments (Fullmer 1958, 1960c). Gingival elastic fibres were differentiated from oxytalan fibres in both the experimental and control tissues. Thus the oxytalan structures retained their identity as a separate fibre component in the metabolically abnormal periodontal ligament.

The unique characteristics of the oxytalan fibres and their long-term maintenance in the midst of experimentally induced abnormal connective tissue, raises speculation as to their physiological role

in the periodontal ligament. The lathyritic state is said to retard the impeded eruption rate of rat incisors (Berkovitz, Migdalski and Solomon 1972; Tsuruta, Eto and Chiba 1974), and also molars (Gardner et al. 1958; Sarnat and Sciaky 1965). Therefore, persistence of the oxytalan meshwork in the lathyritic periodontal ligaments of incisors and molars of young mice, as revealed in the present investigation and also in old lathyritic mice (Sims unpublished observations), suggests that the oxytalan system is not an eruptive guiding system as proposed by Beertsen et al. (1974).

SUMMARY

1. In the lathyritic mouse the oxytalan fibre system of molars and incisors possessed a high degree of permanence.

2. The findings indicated that in the lathyritic mouse the oxytalan fibre was metabolically distinct from elastic and collagen fibres.

3. Experimental lathyrism indicated that the mouse periodontal ligament was a sensitive indicator of the early onset of pathologic changes.

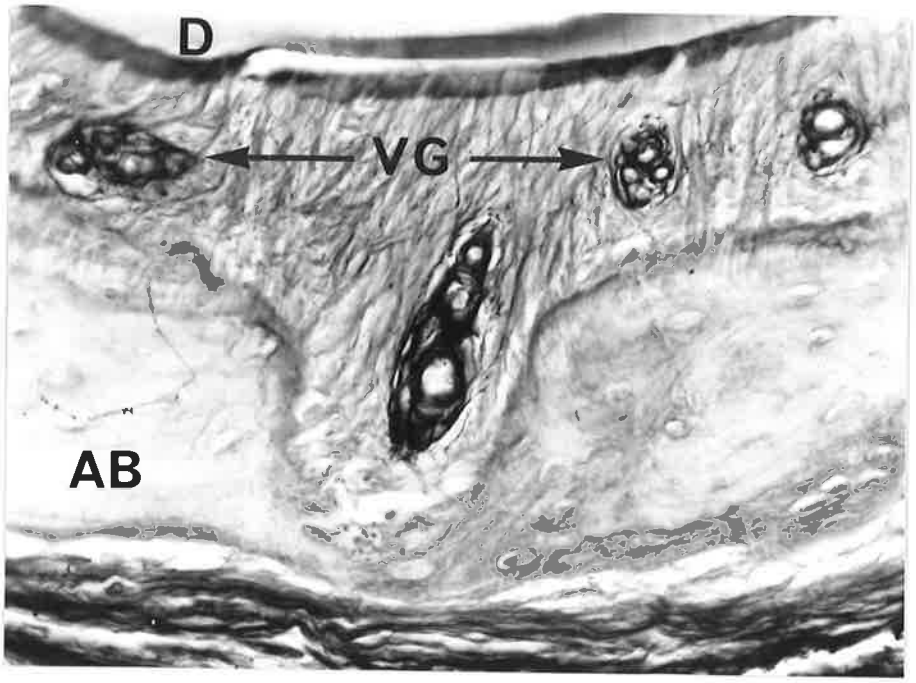

Figure 3.1 Intensified staining of vessels in the buccal periodontal ligament after seven days on a lathyritic diet. Horizontal section opposite the second molar. AB: alveolar bone; D: dentine; VG: vascular group. Oxone, aldehyde fuchsin, light green. X 300

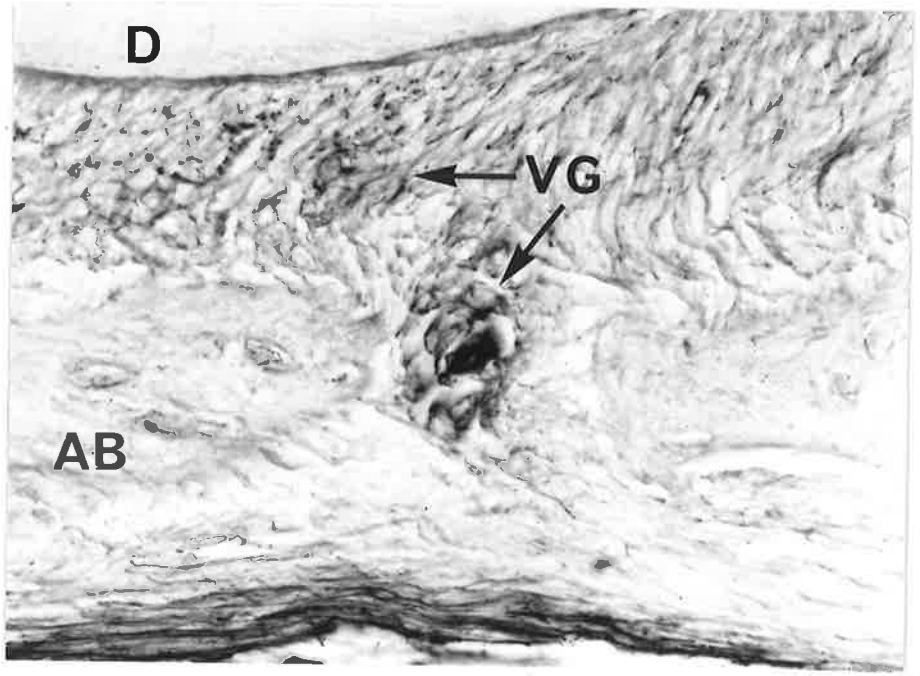

Figure 3.2 Vessel staining in a control section from a normal mouse. Buccal of second molar. AB: alveolar bone; D: dentine; VG: vascular group.
Oxone, aldehyde fuchsin, light green. X 450

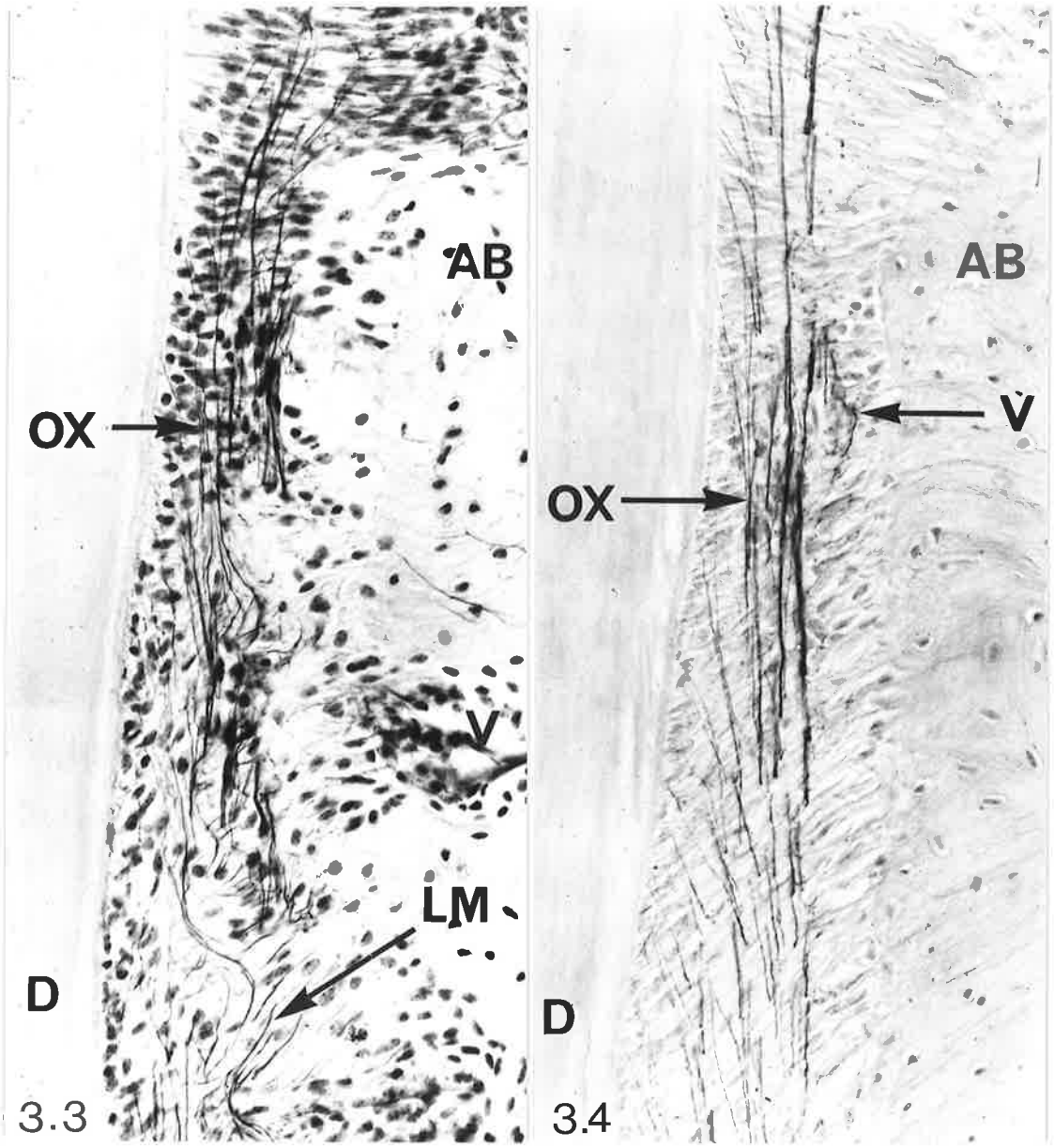

Figure 3.3 Fibre-like lathyritic material in the periodontal ligament after one week on experimental diet. Distal of the first molar. AB: alveolar bone; D: dentine; LM: lathyritic material; OX: oxytalan fibres; V: vessels.
Oxone, orcein. X 360

Figure 3.4 Section of normal periodontal ligament from control mouse. AB: alveolar bone; D: dentine; OX: oxytalan fibres; V: vessels.
Oxone, orcein. X 360

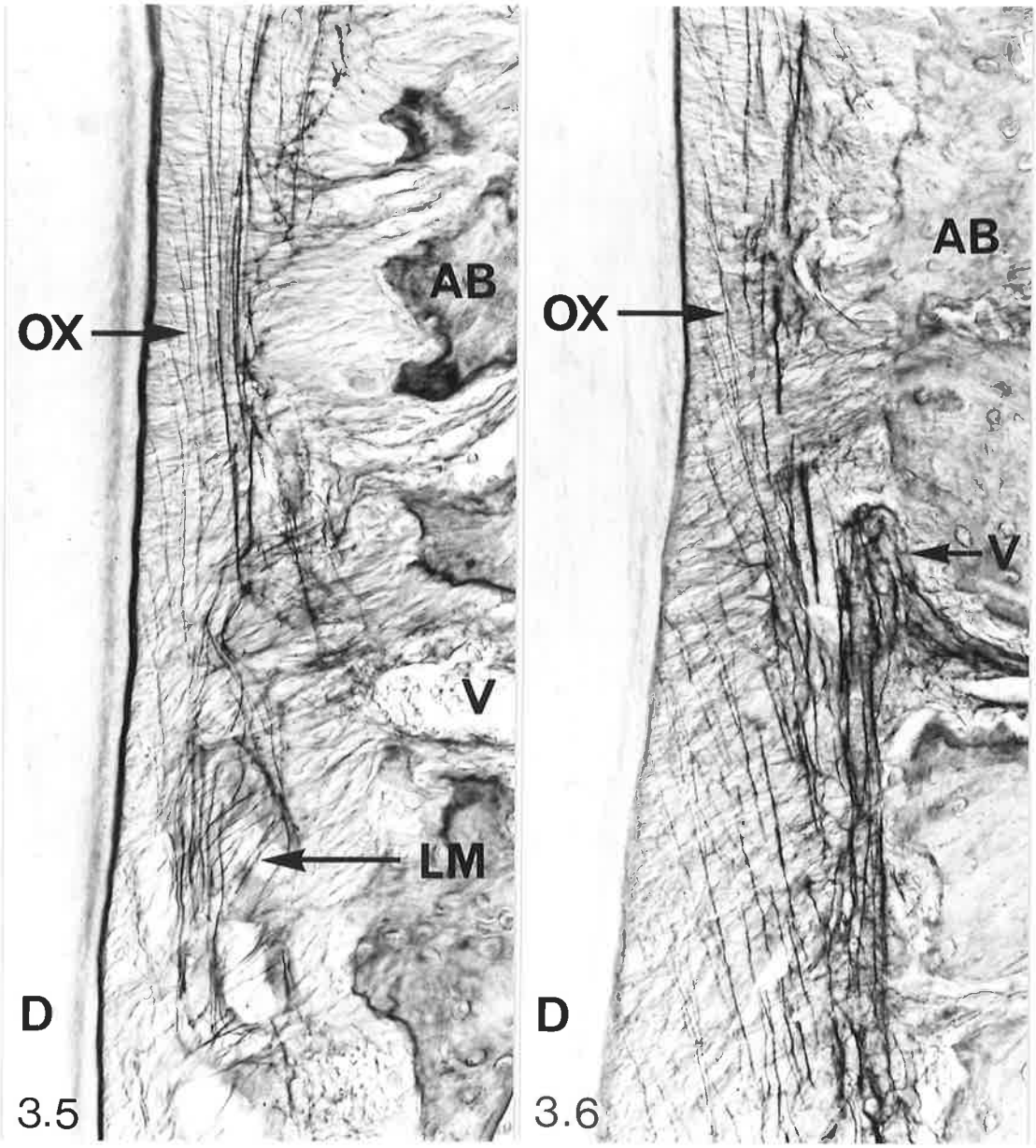

Figure 3.5 Lathyritic material and oxytalan fibres demonstrated with aldehyde fuchsin in a seven day experimental animal. Distal, first molar. AB: alveolar bone; D: dentine; LM: lathyritic material; OX: oxytalan fibres; V: vessel.
Oxone, aldehyde fuchsin, light green. X 360

Figure 3.6 Oxytalan fibre meshwork in the control mouse for Figure 3.5. Distal, first molar. AB: alveolar bone; D: dentine of first molar; OX: oxytalan fibres; V: vessels.
Oxone, aldehyde fuchsin, light green. X 360

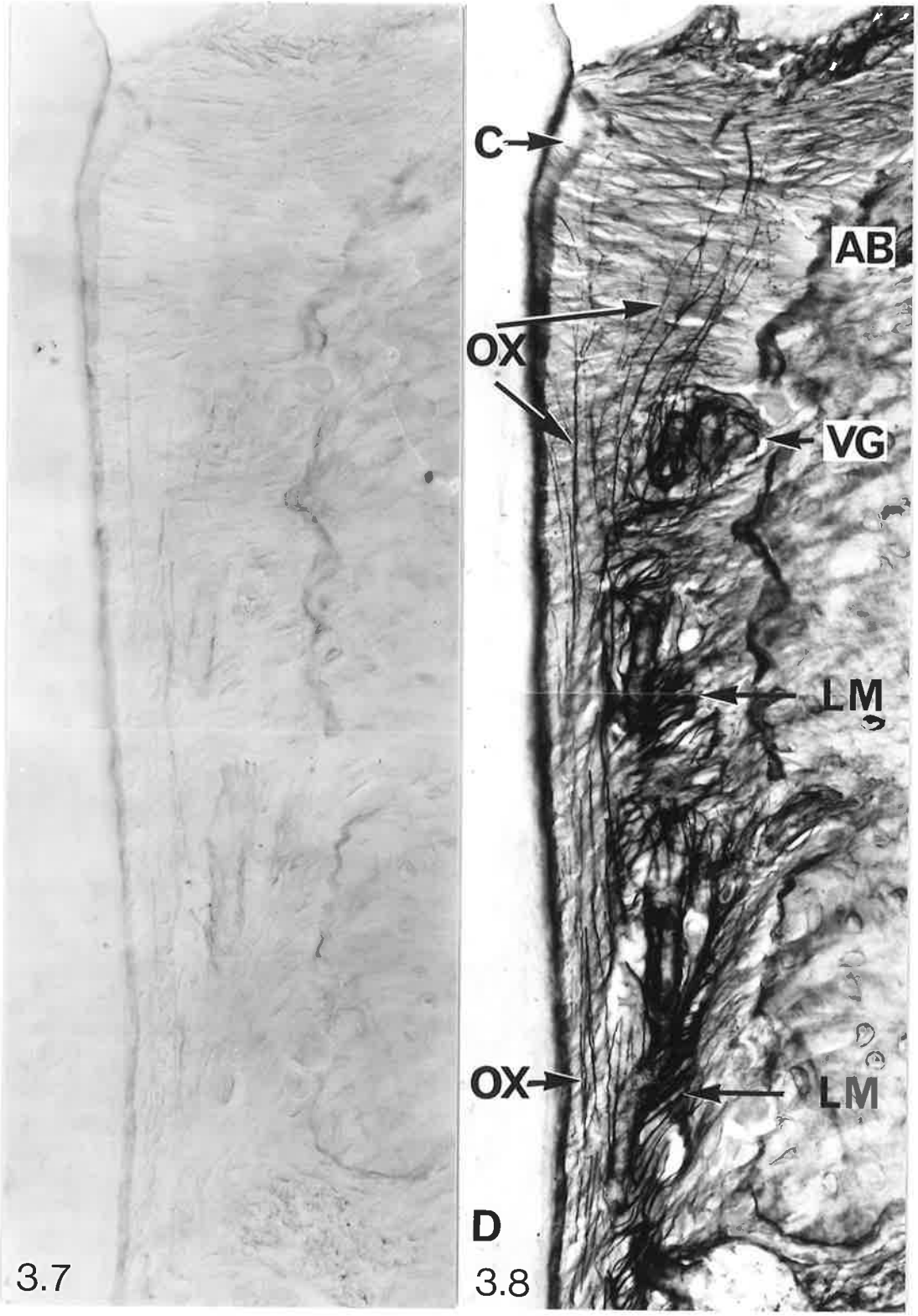

Figure 3.7 Aldehyde fuchsin staining of unoxidized tissue from an eight week lathyritic animal. Mesiodistal section alongside the distal root of a first molar. X 250

Figure 3.8 The section illustrated in Figure 3.7 oxidized and restained with aldehyde fuchsin. Lathyritic material aligned with the principal collagen fibre bundles can be distinguished from oxytalan fibres and the intensely staining vascular groups. AB: alveolar bone; C: cementum; D: dentine; LM: lathyritic material; OX: oxytalan fibres; VG: vascular group. X 250

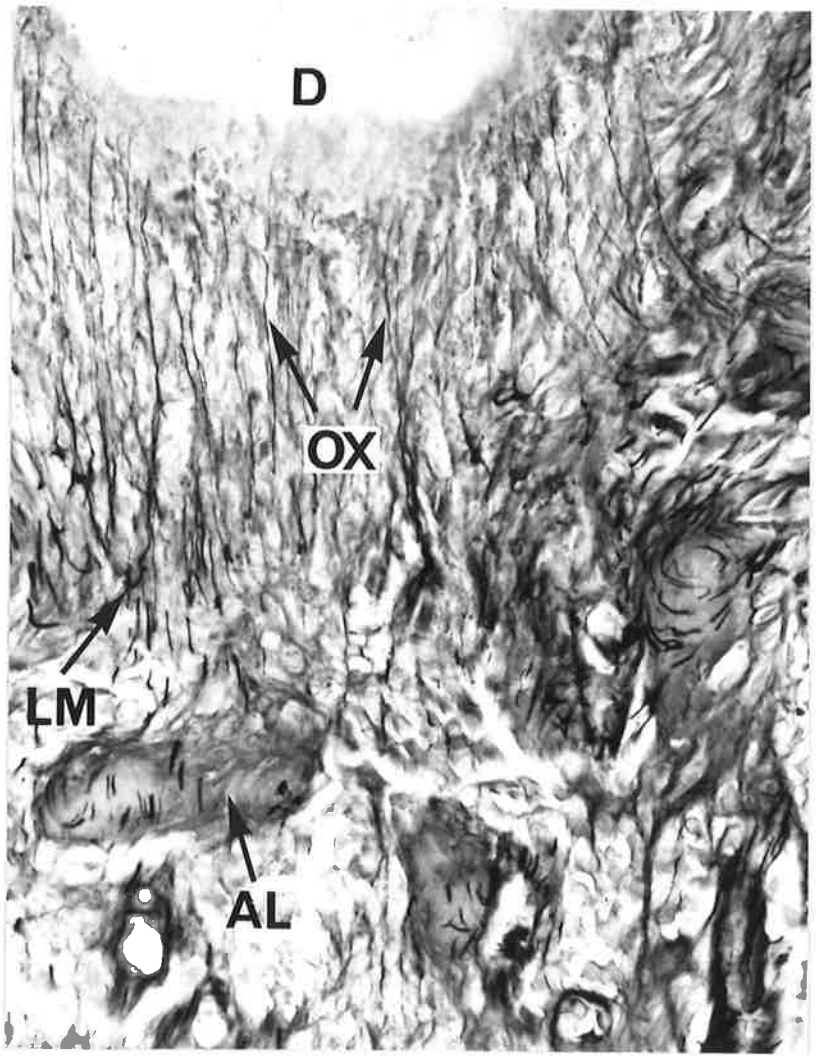

Figure 3.9 Oxytalan fibres, lathyritic material and amorphous lesions in a section tangential to the lingual periodontal ligament of a six week experimental mouse. Amorphous lesions contain lathyritic material. AL: amorphous lesion; D: dentine; LM: lathyritic material; OX: oxytalan fibres.
Oxone, aldehyde fuchsin, light green. X 300

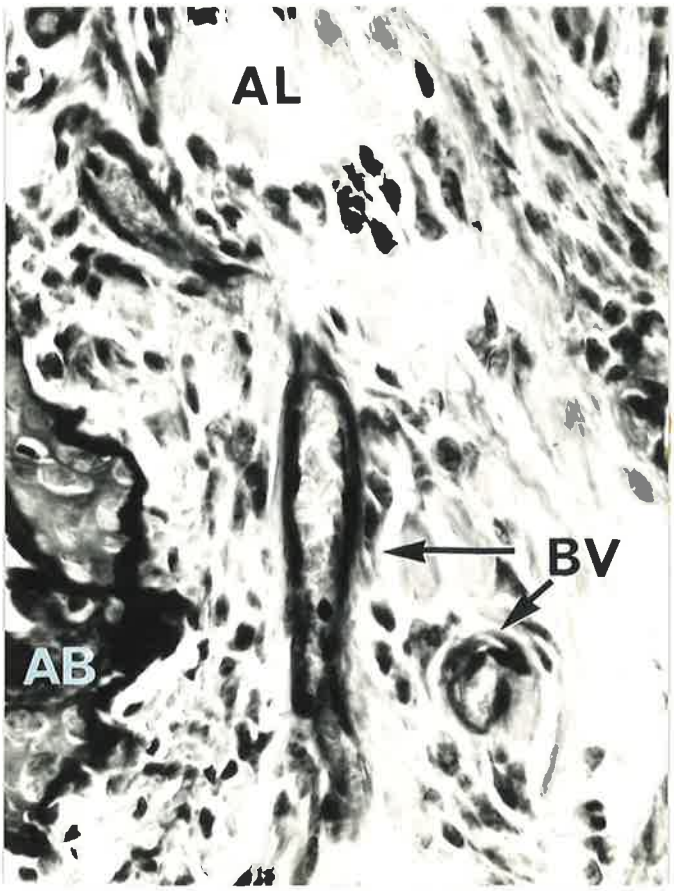

Figure 3.10 Vessel wall stained intensely with PAS after eight weeks of lathyrism. AB: alveolar bone; AL: amorphous lesion; BV: blood vessels.
X 400

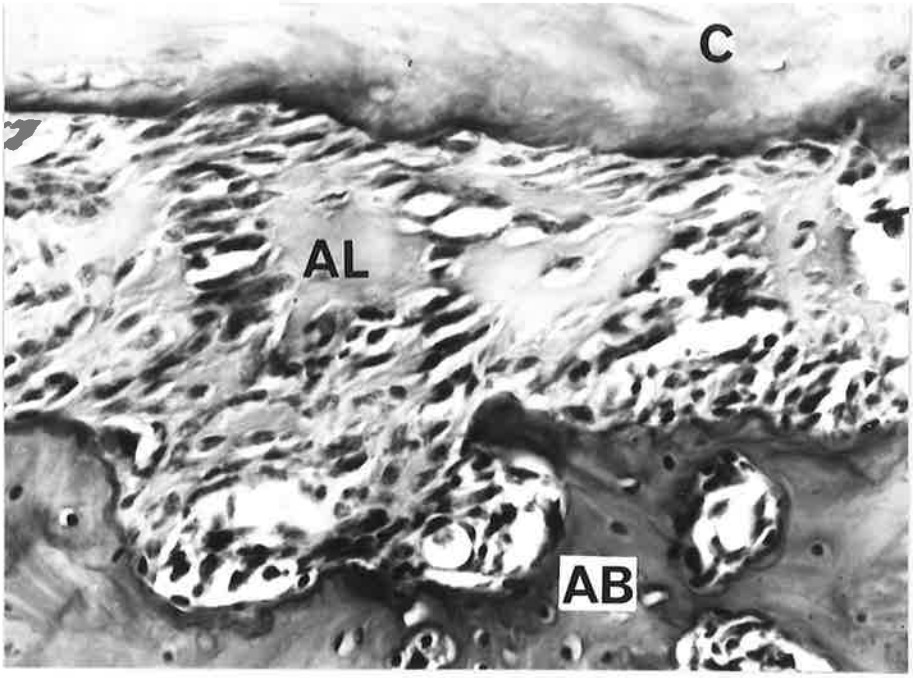

Figure 3.11 Amorphous lesions appear homogeneous when photographed by normal transillumination. Six week lathyritic mouse. AB: alveolar bone; AL: amorphous lesion; C: cementum, distal root of first molar.
PAS. X 300

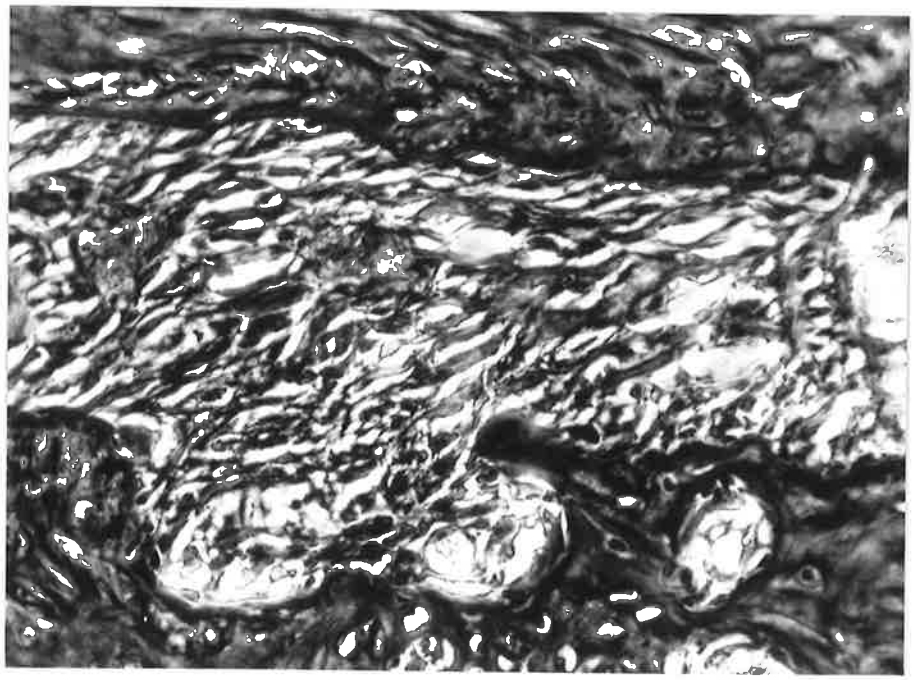

Figure 3.12 Same section as Figure 3.11 viewed under Heine phase contrast illumination shows the fibre-like structure within the amorphous lesions.
X 300

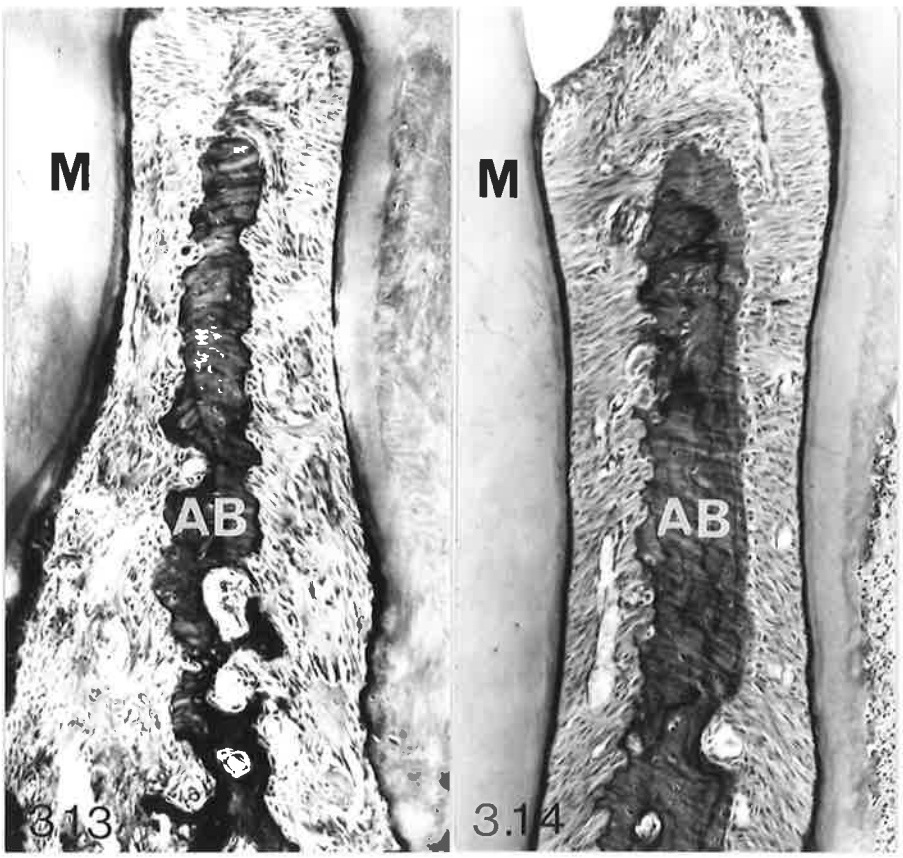

Figure 3.13 Interseptal bone between the first and second molars of a six week lathyritic mouse showing resorption along both mesial and distal surfaces. Many amorphous lesions are present. AB: alveolar bone; M: distal root.
PAS. X 100

Figure 3.14 Distal molar migration in a normal control is associated with resorption on the mesial aspect of the interseptal bone and apposition on the distal surface. AB: alveolar bone; M: distal of first molar.
PAS. X 100

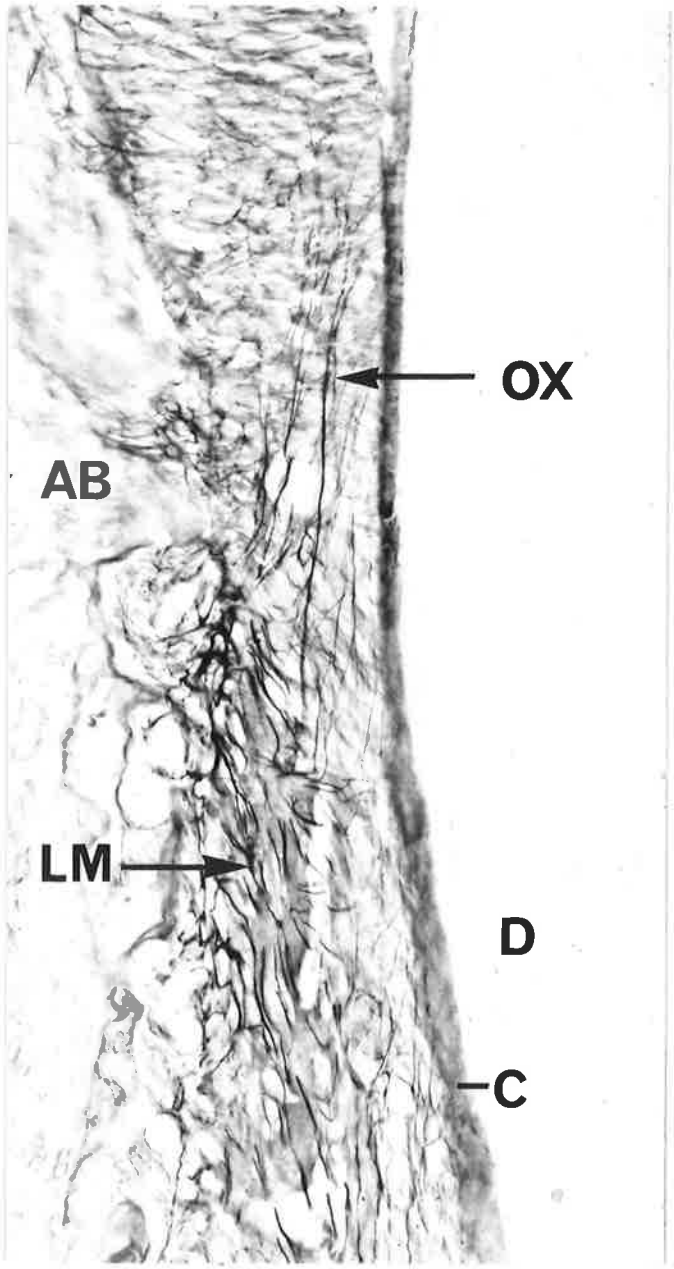

Figure 3.15 Persistence of the oxytalan fibre system after three months of lathyrism. Lathyritic material is associated with amorphous lesions. Distal, first molar. AB: alveolar bone; C: cementum; D: dentine; LM: lathyritic material; OX: oxytalan fibres.
Oxone, aldehyde fuchsin, light green X 375

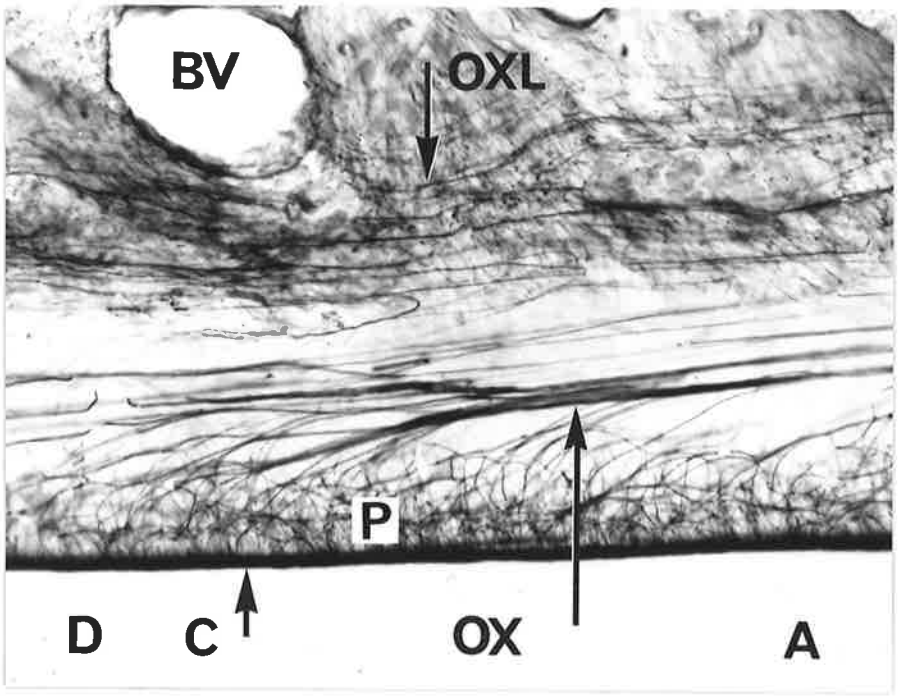

Figure 3.16 Oxytalan fibre system in the incisal periodontal ligament of an eight week lathyritic animal. The three characteristic zones of the mouse oxytalan fibre system remain intact - the plexus, the major oxytalan fibres, and the oxytalan fibres which link the principal vessels. A: apical end of tooth; BV: blood vessel; C: cementum; D: dentine; OX: major oxytalan fibres; OXL: linking oxytalan fibres; P: oxytalan fibre plexus. Oxone, aldehyde fuchsin, light green. X 250

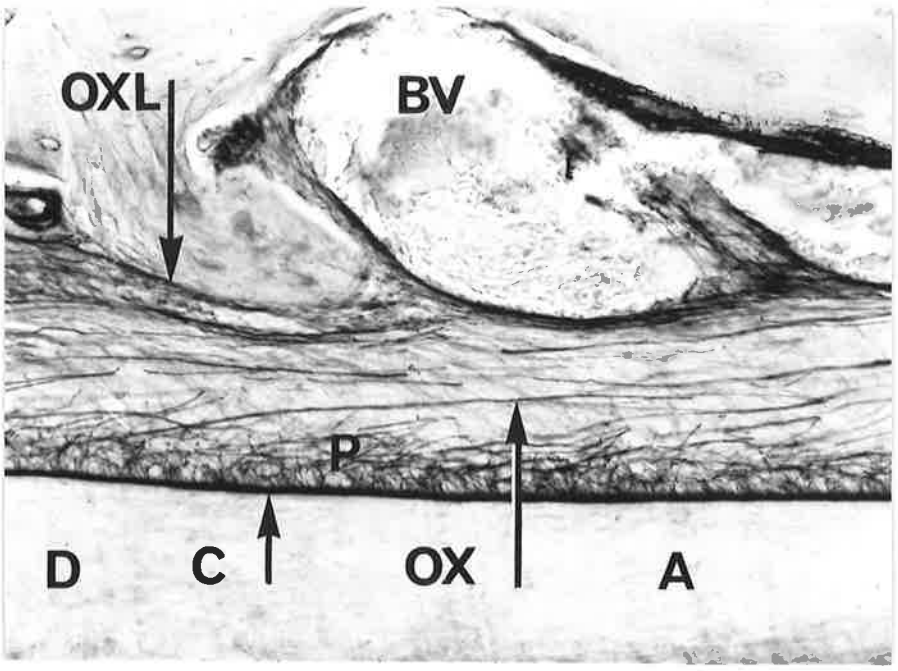

Figure 3.17 Oxytalan fibre system in a control incisor stains less intensely.
Oxone, aldehyde fuchsin, light green. X 300

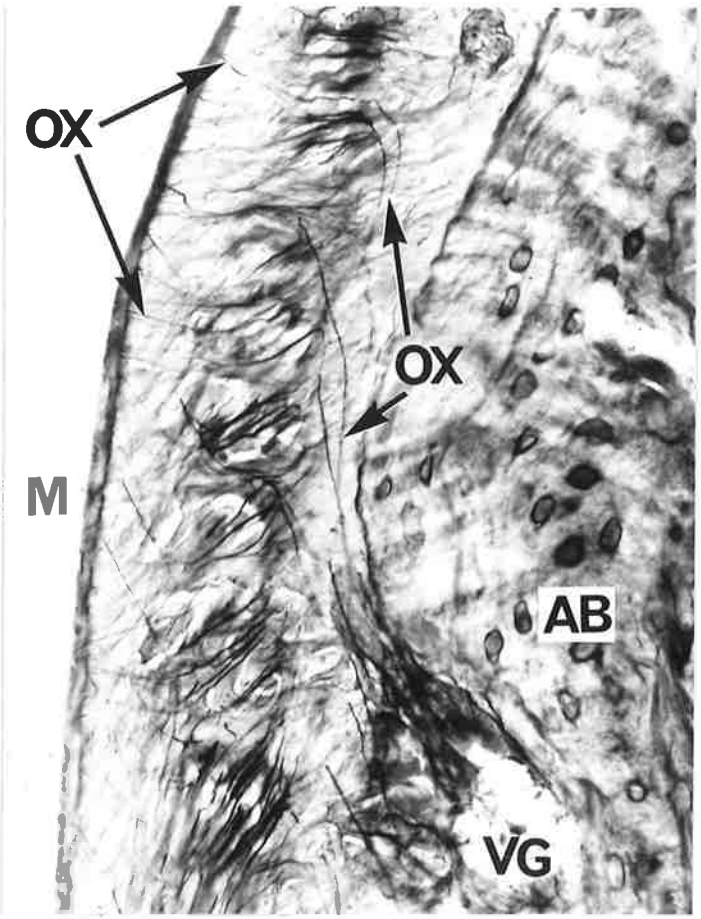

Figure 3.18 Interradicular region of a second molar. Lathyrism has resulted in functional tooth intrusion and apical displacement of that portion of the oxytalan fibres extending between the cementum and middle of the periodontal ligament. AB: alveolar bone; M: mesial root; OX: oxytalan fibres; VG: vascular group.
Oxone, aldehyde fuchsin, light green. X 250

CHAPTER IV

EXPERIMENTAL MOLAR INTRUSION AND EXTRUSION IN NORMAL AND LATHYRITIC MICE

Cause and effect studies of functional changes in the oxytalan fibre system have not been reported. This Chapter describes an investigation which examined the hypothesis (Sims 1973) that the oxytalan fibre system can function independently of other components within the periodontal ligament.

Following experimental molar intrusion or extrusion in normal mice, the angle of attachment of the oxytalan fibre to the cementum surface was measured. The findings were statistically compared with those of duplicate experiments in the pathologically affected periodontal ligament of lathyritic animals where, as the study on lathyrism showed, the oxytalan meshwork remained intact (Sims 1977a) although the tensile strength of the collagen is known to be reduced (Berkowitz, Migdalski and Solomon 1972) resulting in increased tooth mobility.

MATERIALS AND METHODS

After a six month trial period two simple types of spring were designed which could be readily inserted into the mandible and stabilized to produce axial intrusion or extrusion of the mandibular first molar. The first molar was selected because it was the largest and most accessible unit in the molar group.

Male albino mice, aged 21 days, obtained from the Waite

Agricultural Research Institute of the University of Adelaide were selected at random and divided into experimental and control groups. Experimental animals were fed *ad libitum* a lathyritic diet comprising equal parts of ground sweet pea seeds (*Lathyrus odoratus*) and a proprietary brand of mouse cubes (W. Charlick, Adelaide, Australia). Control mice were fed ground mouse cubes *ad libitum*. After one week on their respective diets mice were anaesthetized with the intraperitoneal injection of veterinary Nembutal (Abbot Laboratories, Sydney, Australia) diluted 1 : 19 in physiological saline. Dosage was 0.3 mg of pentobarbitone sodium/3.0 g body weight.

Molar intrusion

Intrusion was produced with the device illustrated in Figure 4.1 which consisted of a small contraction spring formed from .004 mm diameter Special Plus grade orthodontic wire (A.J. Wilcock, Whittlesea, Australia). Welded to one arm was a second arm to provide additional stability during use. After an animal was anaesthetized the lateral surface of the ramus was exposed and the double arms inserted into two No. 7 endodontic reamer holes made parallel and adjacent to the lower border of the mandible. The spring produced an intrusive force of 30 g as estimated prior to insertion with a Correx Tension Gauge (Haag-Streit, A.G., Berne, Switzerland). The gauge had a measuring range of 20-150 g graduated in 5 g increments.

Molar extrusion

Small extrusive springs (Figure 4.1) were formed from .0035 mm diameter Special Plus orthodontic wire. A No. 7 endodontic reamer was used to make a hole through the mandible in the apical region of the first molar. The first hole was made as close to the lower border of

the mandible as practical in order to obviate the risk of jaw fracture during spring insertion. A second hole was made through the alveolus at the bifurcation of the molar with a No. 5 reamer. The spring was then inserted applying an estimated extrusive force of 30 g. Constant intrusion and extrusion times of 30 minutes were employed.

Histological techniques

After positioning the springs the animals were immediately sacrificed and the heads fixed in Lillie's (1965) neutral calcium acetate formalin for 24 h. The mandibles were then dissected out and the soft tissues removed without disturbing the springs. Mandibles with dislodged or imprecisely positioned springs were discarded. Following a further 24 to 48 h in fresh fixative, the jaws were divided at the symphysis, the springs removed and decalcification was commenced. Processing was completed by the double embedding method (Appendix I). Serial sections were cut at 8 μm through the first molar in the mesiodistal axial plane. When histological sections from either side of the mandible proved to be incorrectly angled to the vertical axis of the molar the animal was rejected from the study. Sections fulfilling the required experimental criteria were oxidized in Oxone (Rannie 1963) and stained with a modification of the Fullmer et al. (1974) aldehyde fuchsin technique (Appendix II).

Pilot studies

Preliminary studies were conducted in which ten 28 day old normal male mice, and ten similarly aged mice which had been on a lathyritic diet for one week, were used in each of the following experiments.

Pilot study No. 1

Intrusion:	right first molar	5 normal mice
		and
Control:	left first molar	5 lathyritic mice

Pilot study No. 2

Extrusion:	right first molar	5 normal mice
		and
Control:	left first molar	5 lathyritic mice

Since the mesiodistal sections from some animals were not orientated through the midsagittal plane of the first molar only limited data were obtained for evaluation.

Principal investigation

As the result of information obtained from the pilot study a further series of experiments was undertaken using matching immersion fixation for 90 normal and 90 lathyritic mice, aged 28 days, to compare intrustion and extrusion effects on opposite sides of the mandible in each animal (Figure 4.2). Extrusion and intrusion springs were randomized between left and right sides of the jaw. Forces applied were estimated to be 30 g. Mandibles were processed, sectioned and stained until 25 experimentally acceptable paired left and right sides were obtained from individual mice in both the normal and lathyritic groups. A total of 86 normal and 180 lathyritic blocks were cut. A sample of ten angular readings was taken for each molar tooth.

Method of measuring the attachment angle of oxytalan fibres

Measurements were made of fibre angles in the coronal third of the periodontal ligament alongside the distal of the first molar in

the midsagittal region. This site was selected as it was located in a wide portion of the periodontal ligament where the presence of relatively large oxytalan fibres made their attachment readily observed and measured.

An independent observer measured and recorded the fibres using an Olympus EH binocular microscope with X 10 eyepieces and a X 100 oil immersion objective. One eyepiece had a fixed scale engraved in the optical system. The eyepiece with the scale was placed into the revolving housing on the microscope. To this housing was attached a strip of graph paper. The circumference of the housing equalled 40 divisions of the paper. A reference line was placed on the body of the microscope adjacent to the attached scale. Each division of the graph paper was equivalent to 9 degrees when moved past the reference line. Oxytalan fibre attachment angles were measured by aligning the straight lines of the engraved scale with the estimated direction of the fibre attachment at the cementum and then revolving the eyepiece until the scale was aligned with the cementum surface and estimating the rotation in degrees (Figures 4.3 and 4.4). Although the observer was blind to the source of the tissue sections, he came to recognize the particular drill hole patterns associated with the different types of experimental treatment.

Only those oxytalan fibres which extended into the periodontal ligament for more than 30 μm were assessed. The selected fibres were those located between the cemento-enamel junction and a position 500 μm apically which approximated the coronal third of the ligament. Fibres passing transversely across the interseptal alveolar crest (Sims 1973) were not measured. Where the fibres attached as doublets or triplets all three angles were measured although the

middle fibre tended to be the thickest.

Sources of measurement error included

(i) parallax on the eyepiece revolving scale

(ii) inaccuracy in estimating the scale 1 division = 9^{o}

(This method was equivalent to slide rule estimations)

(iii) assessing the line of the cementum surface and the oxytalan fibre at the site of attachment

Statistical analysis

To provide data for a statistical analysis two conditions not satisfied in the pilot study were included in the principal investigation:

(i) intrusion and extrusion experiments were carried out in each mouse to take out the effect of variation among the mice.

(ii) intrusion and extrusion were randomized between the sides of the mandible from mouse to mouse to prevent a left versus right effect being included in the intrusion versus extrusion effect; i.e. the two types of effect would have been inseparable.

The experimental situation required a mixed model analysis of variance with fixed treatment effects and random mice and mice x treatment effects (Snedcor and Cochran 1967).

The mathematical model used to describe the data was

$$y_{ijk\ell} = \mu + s_i + a_{ij} + t_k + (st)_{ik} + (at)_{ijk} + \epsilon_{ijk\ell} \qquad \begin{matrix} i = 1,2 \\ j = 1,2..25 \\ k = 1,2 \\ \ell = 1,2..10 \end{matrix}$$

where

$y_{ijk\ell}$ the fibre angle measured

$s_i, t_k, (st)_{ik}$ are fixed effects

$a_{ij}, (at)_{ijk}, \varepsilon_{ijk\ell}$ are random effects

$a_{ij} \sim N(0,\sigma_1^2)\ \sigma^2$

$(at)_{ijk} \sim N(0,\sigma_2^2)$

$\varepsilon_{ijk\ell} \sim N(0,\sigma^2)$

μ general mean

s_i effect due to being normal mouse s_1 or lathyritic mouse s_2

a_{ij} measures the contribution of j^{th} animal

$a_{1,1}$ is the first normal mouse

$a_{1,25}$ is the 25^{th} normal mouse

$a_{2,1}$ is the first lathyritic mouse

t_k effect due to treatment from intrusion t_1 or extrusion t_2

$(st)_{ik}$ is an interaction term of the effect of combinations of normal or lathyritic mice with the two types of treatment

$(st)_{1,1}$ is a normal mouse under intrusion

$(st)_{1,2}$ is a normal mouse under extrusion

$(at)_{ijk}$ is an interaction term of the effect of animals with the two types of treatment

ε is the error term

Calculations were performed using the appropriate computer routine from the IMSL FORTRAN collection of functions and subroutines (International Mathematical and Statistical Libraries Inc., Houston, United States).

FINDINGS

In the pilot studies marked compression or widening of the periodontal ligament was evident immediately beneath the apices of intruded or extruded teeth, respectively, when compared with each other and their controls. Many angles of oxytalan fibre attachment appeared to be larger in intruded molars and smaller in extruded molars. These angular changes were most apparent in the cervical region opposite and apical to the alveolar crest. Oxytalan fibres of intruded molars frequently had a more wavy appearance than the fibres of extruded molars which demonstrated a relatively straight, taut appearance. The findings in lathyritic mice were similar. Tearing occurred in the cervical periodontal ligament of two lathyritic extrusion experiments which were, therefore, rejected.

The angular measurements obtained from the pilot studies of molar intrusion and extrusion in normal and lathyritic mice are listed in Tables 4.1 to 4.10 and illustrated as bar graphs in Figure 4.5. These results proved unacceptable for a statistical analysis because of the experimental design and the limited data obtained. Correct experimental design required intrusion and extrusion on opposite sides of the mandible in every mouse, and randomized intrusion and extrusion between left and right sides among mice. Graphs of intrusion and extrusion in individual mice suggested a possible difference in oxytalan fibre angles between intruded and extruded

molars in normal mice. However, bar graphs of the pooled findings for each animal group showed that the distribution of fibre angles differed considerably for intrusion and extrusion in both normal and lathyritic mice. Accordingly, it was decided to repeat the study using a large sample of mice under appropriate experimental conditions to obtain data for an analysis of variance.

Histological assessment of experimental tooth movement in the principal investigations confirmed the findings of the pilot studies. Marked changes were demonstrated in the angle of attachment of major oxytalan fibres to the cementum surface in the coronal region. Molar extrusion in normal animals resulted in generally more acute fibre angles compared with the less acute fibre angles to the cementum of the intruded tooth in the same animal (Figure 4.6). In the apical region a pronounced difference was also observed in the incidence angle of the prominent oxytalan fibres inserting into the cementum of extruded or intruded molars (Figures 4.7 and 4.8). Angular variations were not detected in those portions of the oxytalan fibre meshwork which linked the vessels to each other occluso-apically. Tangential sections through the buccal or lingual periodontal ligament of extruded molars showed that the longer oxytalan fibres were relatively straight as if stretched and under tension (Figure 4.9). However, where fibres were cut obliquely to their principal orientation the resultant short lengths appeared very wavy as if they had rebounded after being stretched and divided while under tension. As a contrast, similar sections of intruded molars showed that the majority of oxytalan fibres were relatively wavy as if they had been able to relax along their course between the cementum and the blood vessels (Figure 4.10).

Experiments in lathyritic mice also showed a marked difference between the magnitude of the oxytalan fibre angles in the intruded and extruded molars (Figure 4.6). However, the fragile condition of the lathyritic tissues resulted in minute tears in the interproximal periodontal ligament region of many mice during the insertion of extrusion springs. These tears were not detectable until the tissues were processed and stained when the affected mice were eliminated from the study.

Data of the 10 oxytalan fibre angle readings from each molar tooth are summarized by averages in Tables 4.11 and 4.12. Differences in fibre angulation resulting from extrusion and intrusion varied from approximately 16 to 66 degrees in normal mice and from 11 to 79 degrees in lathyritic mice. The analysis of variance for the normal and lathyritic group is shown in Table 4.13. There were significant effects due to species, treatments, and species x treatments at the 5% level.

DISCUSSION

Histological assessment of experimental tooth movement in the mouse resulted in microscopically evident and statistically significant changes in the angle of attachment of oxytalan fibres to the cementum surface in the coronal third of the periodontal ligament. Spatial changes in the relationship of the oxytalan fibre meshwork were also discerned in the apical region of the periodontal ligament although changes in this region were not analysed statistically. Experimentally induced changes were particularly evident at the cervical and apical extremes of the root but also occurred throughout the oxytalan fibre meshwork and at its attachment to the cementum.

The waviness of short portions of major oxytalan fibres in the extrusion experiments indicated that these fibres had recoiled because of loss of support along their length from anastomoses with other major oxytalan fibres and the fine intercommunicating oxytalan fibres (Sims 1975). Fullmer et al. (1974) state that the oxytalan fibre appears to possess unique properties yet shows similarities with elastic fibres. Therefore, it is tempting to speculate that in the highly specialized functional requirements of the mammalian periodontal ligament, the oxytalan fibre may represent the development of a structural protein elastomer which provides nearly perfect mechanical springs where complete and instantaneous elastic recovery is required (Weis-Fogh 1960). Moreover, the histological evidence that oxytalan fibres are capable of being stretched and relaxed supports the author's belief that the oxytalan fibre meshwork normally exists in a state of prestress like other fibre components in the body (Tkaczuk 1968; Crisp 1972).

Comparatively heavy axial loads applied to molars of normal and lathyritic mice did not produce evidence of a generalized collapse of blood vessels within the periodontal ligament. Castelli and Dempster (1965) also report lack of vascular collapse when loads are applied to the incisors of normal monkeys. Evidence that tooth intrusion in both the normal and pathologically affected periodontal ligament of mice was associated with relaxation of oxytalan fibres without vascular occlusion suggested that the oxytalan fibre meshwork did not provide mechanical support for the maintenance of vascular patency as proposed by Rannie (1963) and Carmichael (1968).

Data in the statistical analysis demonstrated the fact that real differences were due to whether a mouse was normal or lathyritic,

and whether a molar was intruded or extruded. A real interaction also occurred between the species and the type of treatment applied which was just significant at the 5% level. These results may be interpreted on the basis of histological observations and reference to Tables 4.11 and 4.12 where real differences in mean oxytalan fibre angles could be attributed to anatomical factors, and the reduced tensile strength of collagen in the lathyritic periodontium. In histological sections intrusion springs caused more compression of the periodontal ligament below the root apices of lathyritic mice than normal mice. Since the applied 30 g force was relatively strong for the size of a mouse, it was concluded that distortion of the lathyritic weakened periodontal structures, including the alveolar bone, accounted for the increased average fibre angle compared with that in the normal mouse. Extrusion of molars produced with the same force was anatomically unimpeded and frequently resulted in rupture of the lathyritic periodontal ligament. Comparison of mean fibre angles in normal and lathyritic mice supported the histological observations that in lathyritic mice little tooth extrusion occurred beyond that seen in normal mice before the weakened lathyritic periodontal ligament ruptured. The reduced tensile strength of lathyritic collagen was confirmed by the ease with which mice on a lathyritic diet for seven days could be rendered edentulous.

Preparation of tissues for routine histologic examination and evaluation results in a certain amount of shrinkage and some distortional changes (Smith 1962). While recognizing the influence of such factors in the present study it is considered that the statistically significant differences demonstrated in the magnitude of the oxytalan fibre angles represented real effects produced in vivo and were not the consequences of tissue alterations associated with

processing techniques.

It is appreciated that the experimental loads applied to the molars were of different functional and physiological significance to those normally derived from vascular pulsation (Körber 1970) and the momentary forces of mastication (Bien 1966). Nevertheless, under the experimental conditions employed, which amplified the range of normal tooth oscillation, the findings suggested that different regions of the oxytalan fibre meshwork were capable of some degree of independent functional variation from the collagen fibre system, cells, the extracellular substance, vessels and nerves.

On the basis of the data presented this investigation provides additional information on the response of the periodontal ligament to tooth loading and lends support to the hypothesis (Sims 1973, 1974, 1976) that the oxytalan fibre system is capable of some degree of independent function within the periodontal ligament.

SUMMARY

1. Experimental tooth movement in normal and lathyritic mice demonstrated histologically evident and statistically significant changes in the angle of oxytalan fibre attachment to the cementum surface.

2. Statistically significant changes occurred due to species (normal or lathyritic), treatments (intrusion or extrusion), and there was a species by treatment interaction.

3. Tissue sections provided static evidence of oxytalan fibre extension and relaxation.

TABLE 4.1

Fibre angles from the normal mouse providing the maximum number of measurements in the pilot intrusion study No. 1.

	Intrusion: Right Molar Mouse (MRS M48A)			Control: Left Molar Mouse (MRS M48B)		
Fibre angles (degrees)	Fibre angles recorded	Number of fibres	% of total fibres	Fibre angles recorded	Number of fibres	% of total fibres
11-15						
16-20	20	1	5			
21-25						
26-30	30	1	5	30,30	2	14
31-35	35	1	5	35,35	2	14
36-40	40,40,40	3	15	40,40	2	14
41-45	45,45,45,45	4	20	45,45	2	14
46-50	50,50,50	3	15	50,50,50	3	21
51-55	55,55	2	10	55	1	7
56-60	60,60,60	3	15			
61-65	65,65	2	10	65,65	2	14
66-70						
71-75						
76-80						
81-85						
86-90						
	n = 20 observations			n = 14 observations		

TABLE 4.2

Fibre angle distribution from the normal mouse providing the maximum number of measurements in the pilot extrusion study No. 2.

Extrusion: Right Molar Mouse (MRS M7A)				Control: Left Molar Mouse (MRS M7B)		
Fibre angles (degrees)	Fibre angles recorded	Number of fibres	% of total fibres	Fibre angles recorded	Number of fibres	% of total fibres
11-15	15,15,15	3	23	15,15	2	12
16-20	20,20,20	3	23	20,20	2	12
21-25	25,25,25	3	23	25,25,25,25	4	24
26-30	30,30,30	3	23	30,30,30	3	18
31-35	35	1	8	35,35	2	12
36-40				40,40	2	12
41-45				45	1	6
46-50				50	1	6
51-55						
56-60						
61-65						
66-70						
71-75						
76-80						
81-85						
86-90						
n = 13 observations				n = 17 observations		

TABLE 4.3

Total of 65 angular measurements from intruded right molars of 4 normal mice in pilot study No. 1.

Mouse (MIEL)	No. 1		No. 2		No. 3		No. 4		No. 5
Histo-logical section	23a	23b	27a	27b	32a	32c	35a	35b	
Fibre Angles (degrees)	30 42 50 70 78 80 81	48 62 65 70	40 45 53 53 54 56	30 50 50 54 55 60 60 75 80 80 85 90	25 40 46 50 52 59 75 80 81	27 28 34 35 38 38 46 72 73	25 27 30 31 35 42 45 50 55	25 40 43 45 45 50 54 55 65	REJECT

TABLE 4.4

Fibre angle distribution of intruded molars of the 4 normal mice in pilot study No. 1.

Fibre Angles (degrees)	Mouse (MIEL)					No. of fibres	% of total fibres
	No. 1	No. 2	No. 3	No. 4	No. 5		
11-15							
16-20							
21-25			1	2		3	5
26-30	1	1	2	2		6	9
31-35	0	0	2	2		4	6
36-40	0	1	3	1		5	8
41-45	1	1	0	5		7	11
46-50	2	2	3	2	REJECT	9	14
51-55	0	5	1	3		9	14
56-60	0	3	1	0		4	6
61-65	2	0	0	1		3	5
66-70	2	0	0			2	3
71-75	0	1	3			4	6
76-80	2	2	1			5	8
81-85	1	1	1			3	5
86-90	0	1	0			1	2

TABLE 4.5

Total of 54 angular measurements from extruded right molars of 4 normal mice in pilot study No. 2.

Mouse (MIER)	No. 1	No. 2	No. 3	No. 4	No. 5
Histo-logical Section	27b		18a 18b	30a 30b	28b
Fibre Angles (degrees)	25 25 28	REJECT	24 14 28 18 30 20 27 30 32 32 34 35 36 37 17a 17b 17c 20 18 18 22 22 25 24 24 30 25 25 40 38 38 42	18 28 20 28 25 32 25 35 25 40 25 45 28 50 28 30 35 40	22 27 36 48

TABLE 4.6

Fibre angle distribution of extruded molars of the 4 normal mice in pilot study No. 2

Fibre Angles (degrees)	Mouse (MIER)					No. of fibres	% of total fibres
	No. 1	No. 2	No. 3	No. 4	No. 5		
11-15			1			1	2
16-20			5	2		7	13
21-25	2		8	4	1	15	28
26-30	1		5	5	1	12	22
31-35			4	3	0	7	13
36-40			5	2	1	8	15
41-45		REJECT	1	1	0	2	4
46-50				1	1	2	4
51-55							
56-60							
61-65							
66-70							
71-75							
76-80							
81-85							
86-90							

TABLE 4.7

Total of 42 angular measurements from intruded right molars of 4 lathyritic mice in pilot study No. 1.

Mouse (LMIEL)	No. 1		No. 2	No. 3		No. 4		No. 5
Histo-logical section	18a	19a	23b	30b	31a	35a	35b	
Fibre Angles (degrees)	26	32	25	35	30	29	27	REJECT
	35	36	25	40	34	31	30	
	53	50	30	42	40	33	45	
	62		35	45	45	34	52	
	65		40	45	50	36	60	
	70		40		60	45	80	
			45			45		
			60			45		

TABLE 4.8

Fibre angle distribution of intruded molars of the 4 lathyritic mice in pilot study No. 1.

Fibre Angles (degrees)	Mouse (LMIEL)					No. of fibres	% of total fibres
	No. 1	No. 2	No. 3	No. 4	No. 5		
11-15							
16-20							
21-25		2				2	5
26-30	1	1	1	3		6	14
31-35	2	1	2	3		8	19
36-40	1	2	2	1		6	14
41-45	0	1	4	4	REJECT	9	21
46-50	1	0	1			2	5
51-55	1	0	0	1		2	5
56-60	0	1	1	1		3	7
61-65	2					2	5
66-70	1					1	2
71-75	0					0	
76-80				1		1	2
81-85							
86-90							

TABLE 4.9

Total of 21 angular measurements from extruded right molars of 3 lathyritic mice in pilot study No. 2.

Mouse (LMIER)	No. 1		No. 2	No. 3	No. 4	No. 5	
Histo-logical section	29a	29b			40a	31a	
Fibre Angles (degrees)	26	22	REJECT	REJECT	35	25	
	28	33			36	26	
	30	42				27	
						28	
	30a	30b				32a	32b
	22	14				12	22
	24	18					23
	26	20					

TABLE 4.10

Fibre angle distribution of extruded molars of the 3 lathyritic mice in pilot study No. 2.

Fibre Angles (degrees)	Mouse (LMIER)					No. of fibres	% of total fibres
	No. 1	No. 2	No. 3	No. 4	No. 5		
11-15	1				1	2	10
16-20	2				0	2	10
21-25	3				3	6	29
26-30	4				3	7	33
31-35	1	REJECT	REJECT	1		2	10
36-40	0			1		1	5
41-45	1					1	5
46-50							
51-55							
56-60							
61-65							
66-70							
71-75							
76-80							
81-85							
86-90							

TABLE 4.11

Normal mice: Average of 10 angular readings for each mouse-treatment combination

Mouse	Intrusion	Extrusion
1	64.8	18.0
2	62.0	31.5
3	59.4	21.8
4	61.6	33.2
5	65.7	18.2
6	56.3	18.6
7	66.1	16.0
8	61.1	18.9
9	59.1	20.9
10	57.3	27.5
11	56.3	23.1
12	46.7	22.2
13	49.1	19.6
14	49.7	18.2
15	50.7	16.5
16	57.8	19.0
17	47.3	30.4
18	56.9	20.2
19	47.4	24.8
20	53.9	21.9
21	59.8	19.9
22	55.8	24.9
23	62.8	18.3
24	65.7	32.4
25	66.2	21.3
Average over mice	57.6	22.3

TABLE 4.12

Lathyritic mice: Average of 10 angular readings for each mouse-treatment combination

Mouse	Intrusion	Extrusion
1	60.4	33.7
2	52.0	30.0
3	44.9	25.7
4	53.2	30.3
5	50.2	23.1
6	71.0	18.3
7	67.0	26.8
8	64.4	25.9
9	62.3	19.5
10	64.3	35.4
11	59.4	22.8
12	58.0	19.8
13	76.8	21.2
14	76.0	11.2
15	72.4	26.8
16	79.3	26.5
17	76.4	16.2
18	67.7	17.0
19	60.4	37.2
20	72.2	19.2
21	69.2	18.3
22	60.6	17.9
23	61.5	17.6
24	76.6	19.5
25	77.6	26.4
Average over mice	65.3	23.4

TABLE 4.13

Analysis of variance for total sample of normal and lathyritic mice

Source	DF	SS	MS	Ratio
Species	1	4986.29	4986.29	13.12***
Animals within Species	48	18241.00	380.02	2.88
Treatments	1	372374.21	372374.21	618.18***
Species x Treatments	1	2732.41	2732.41	4.54*
Animals x Treatments	48	28913.83	602.37	4.57
Error	900	118652.70	131.84	
Total	999	545900.44		

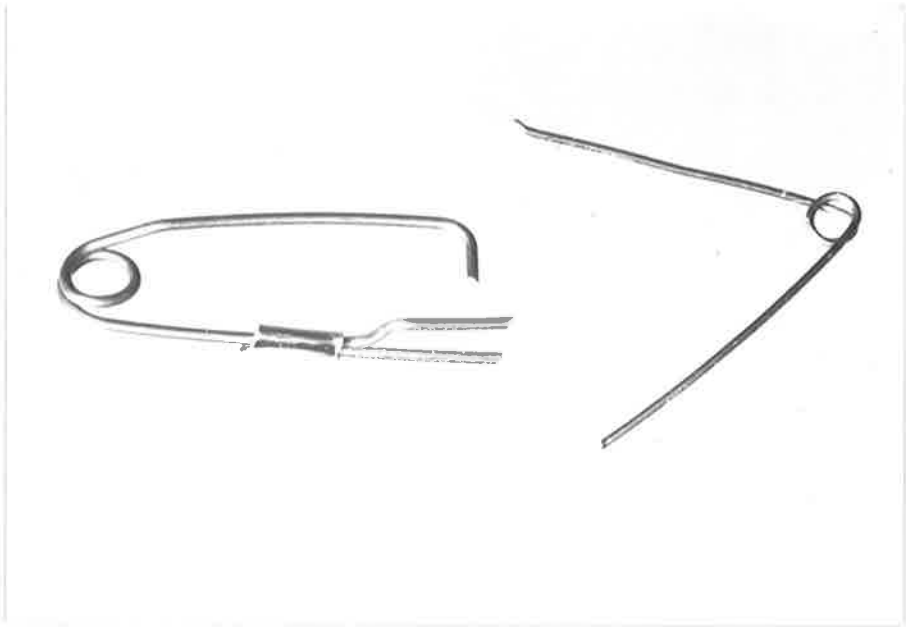

Figure 4.1 Intrusion spring (left) and extrusion spring (right).

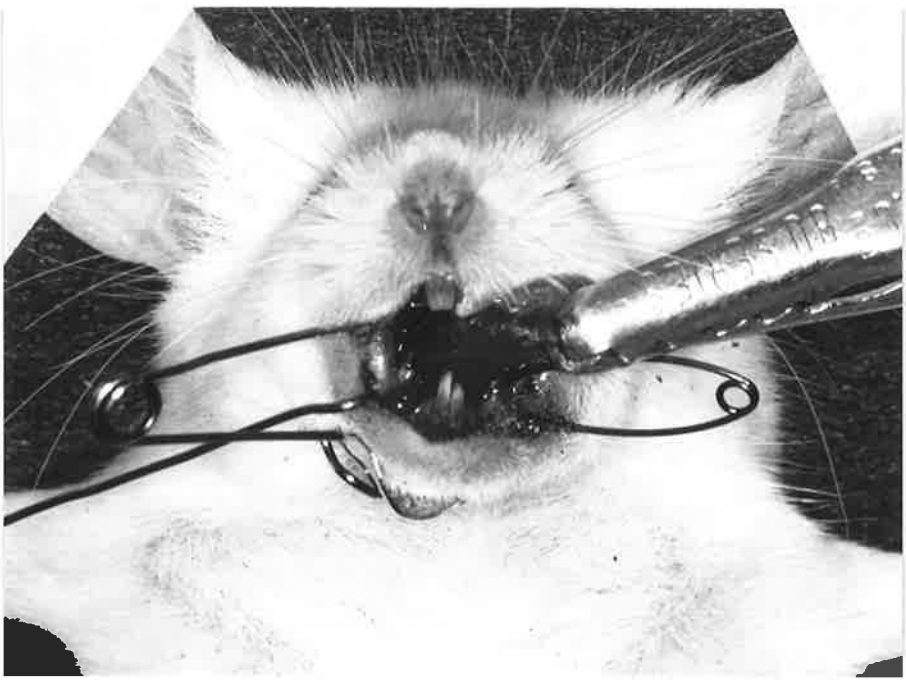

Figure 4.2 Anaesthetized mouse with intrusion spring (left) and extrusion spring (right) inserted in the mandible.

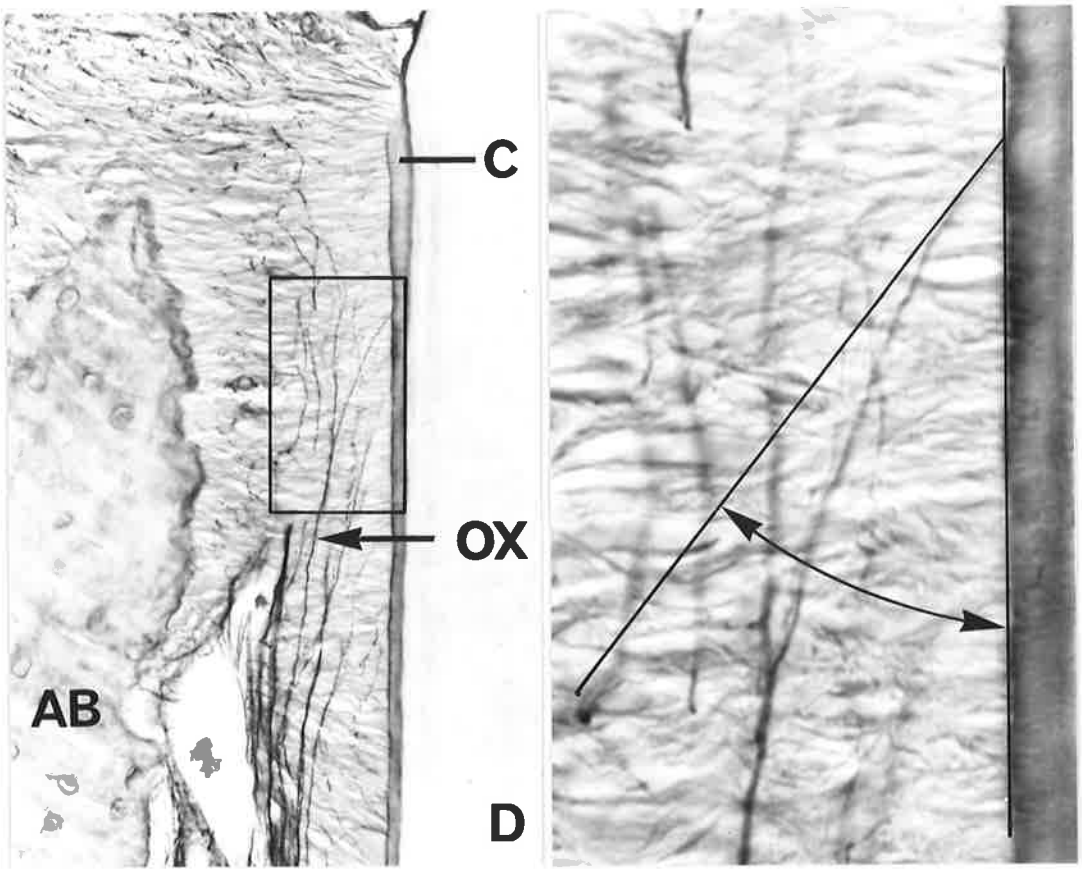

Figure 4.3 Region on the distal of an extruded first molar where the angle of oxytalan fibre attachment was measured. AB: interseptal alveolar bone; C: cementum; D: dentine; OX: oxytalan fibre. Oxone, aldehyde fuchsin, light green. X 250

Figure 4.4 Enlargement of the region outlined in Figure 4.3 showing the angle measured. X 1000

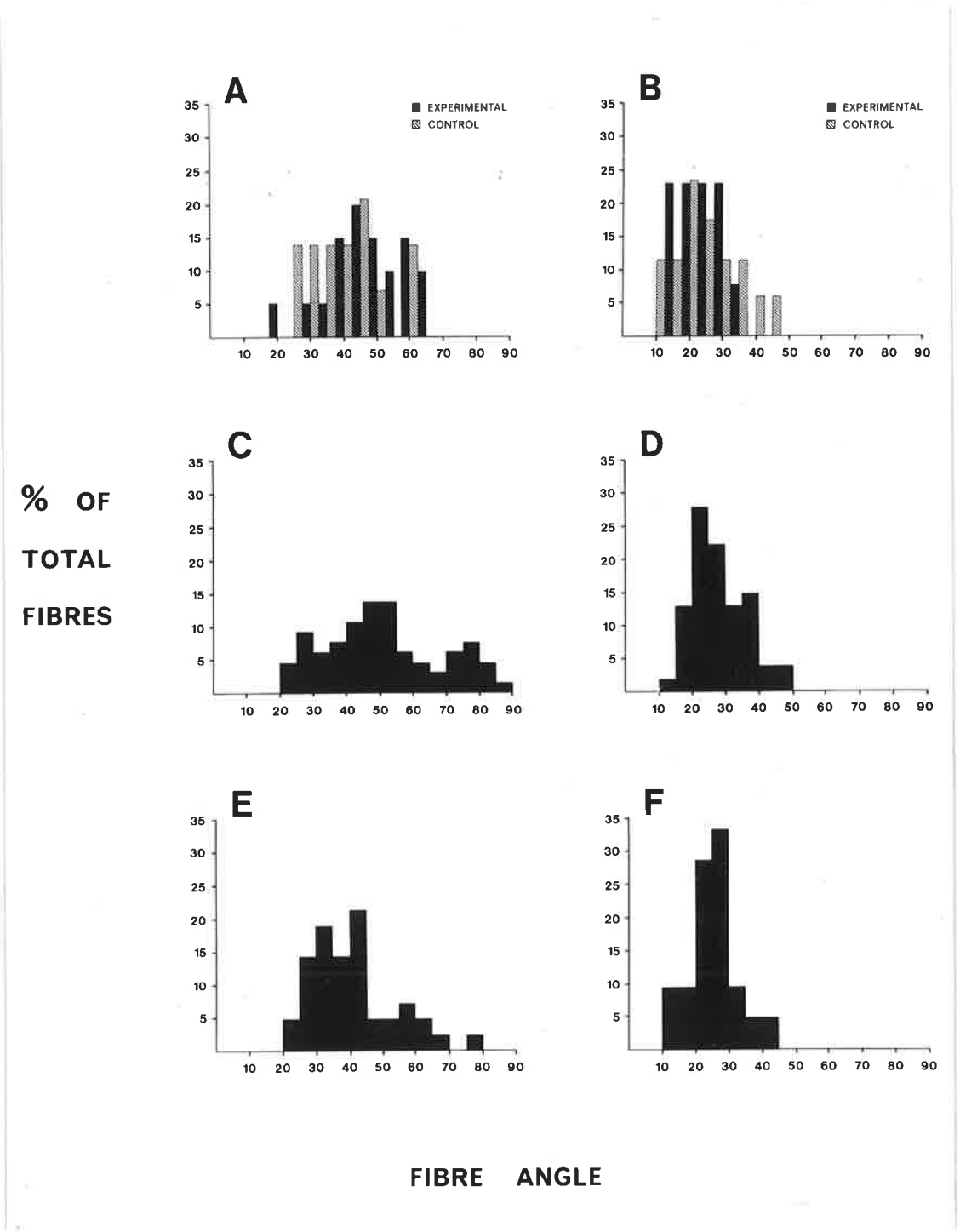

Figure 4.5 Total angular measurements from each pilot study presented as bar graphs.

A. Molar intrusion and control molar in a normal mouse
B. Molar extrusion and control molar in a normal mouse
C. Molar intrusion in 4 normal mice
D. Molar extrusion in 4 normal mice
E. Molar intrusion in 4 lathyritic mice
F. Molar extrusion in 3 lathyritic mice

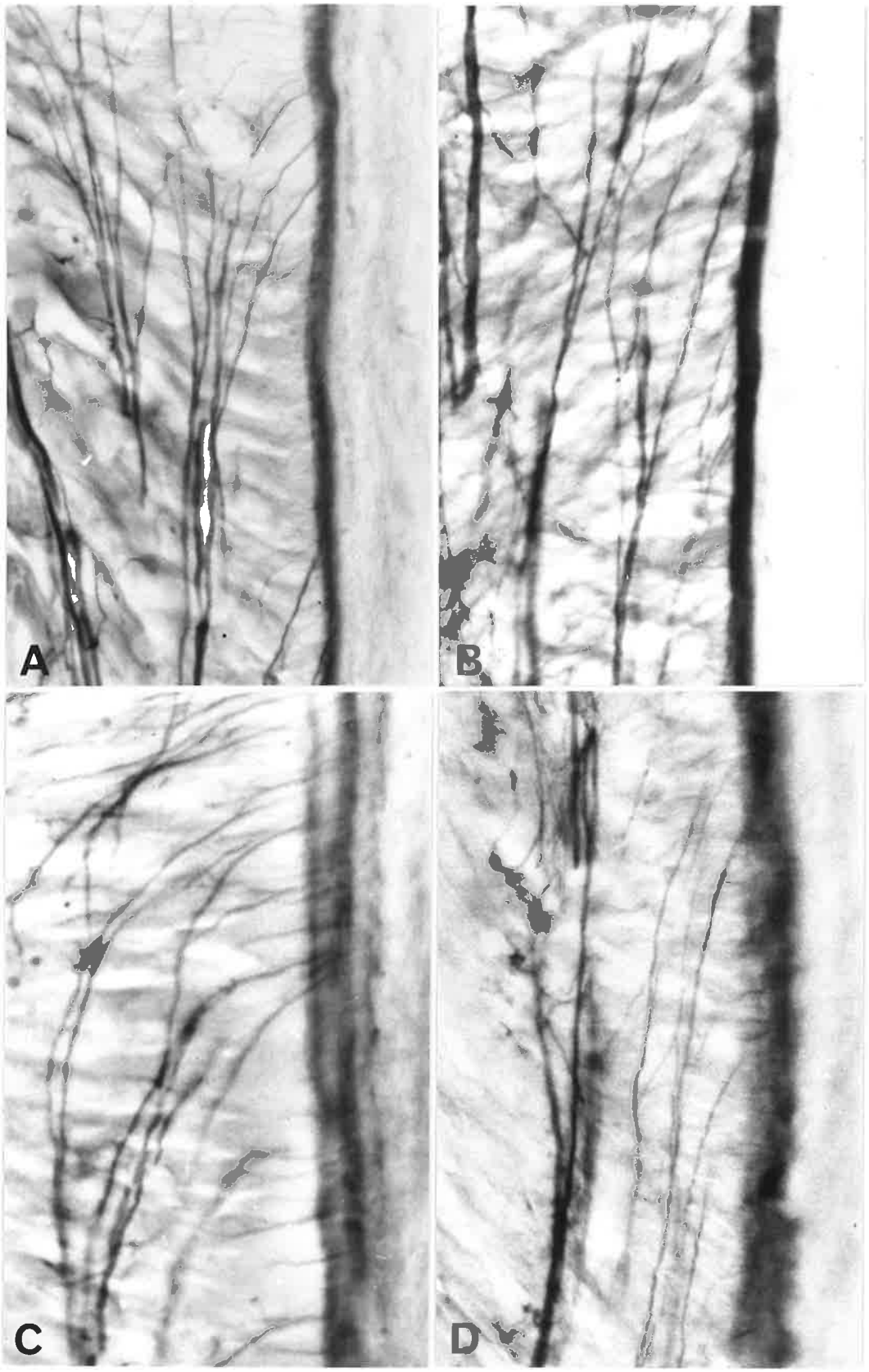

Figure 4.6 Oxytalan fibre angles resulting from different treatments.

A. Intrusion in a normal mouse
B. Extrusion in a normal mouse
C. Intrusion in a lathyritic mouse
D. Extrusion in a lathyritic mouse

Oxone, aldehyde fuchsin, light green. X 1000

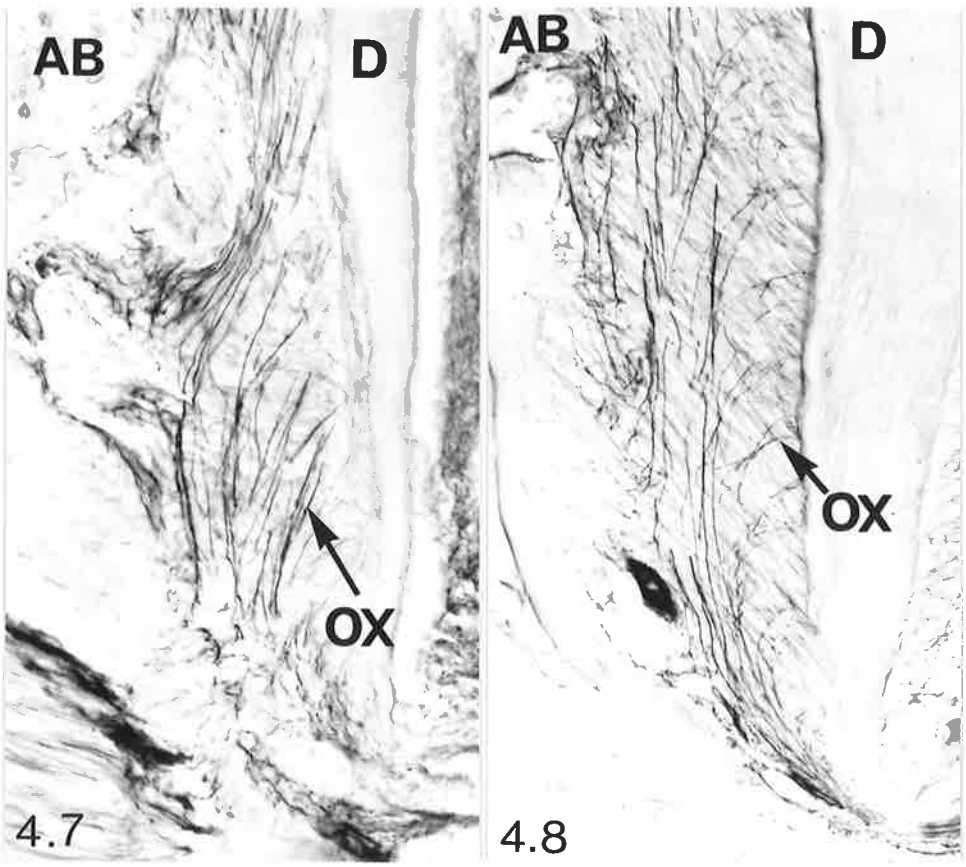

Figure 4.7 Oxytalan fibre angles to the root surface of an extruded molar. Apex of distal root.
AB: alveolar bone; D: dentine; OX: oxytalan fibres.
Oxone, aldehyde fuchsin, light green. X 250

Figure 4.8 Oxytalan fibre relationship to the root surface of an intruded molar. Apex of distal root.
AB: alveolar bone; D: dentine; OX: oxytalan fibres.
Oxone, aldehyde fuchsin, light green. X 250

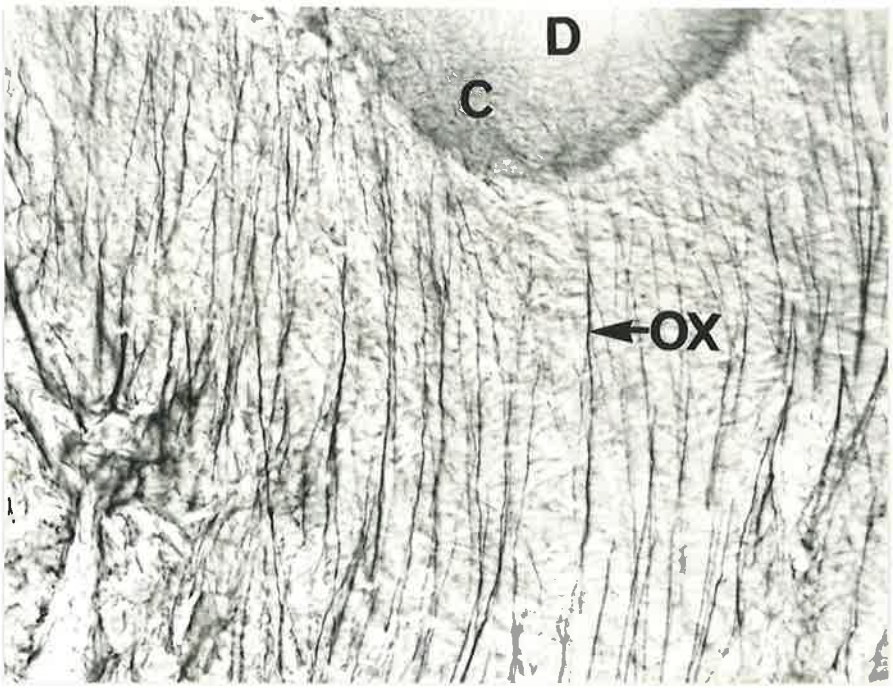

Figure 4.9 Oxytalan fibres in the cervical region of an extruded tooth. Normal mouse. Buccal tangential section. C: cementum; D: dentine; OX: oxytalan fibres.
Oxone, aldehyde fuchsin, light green. X 450

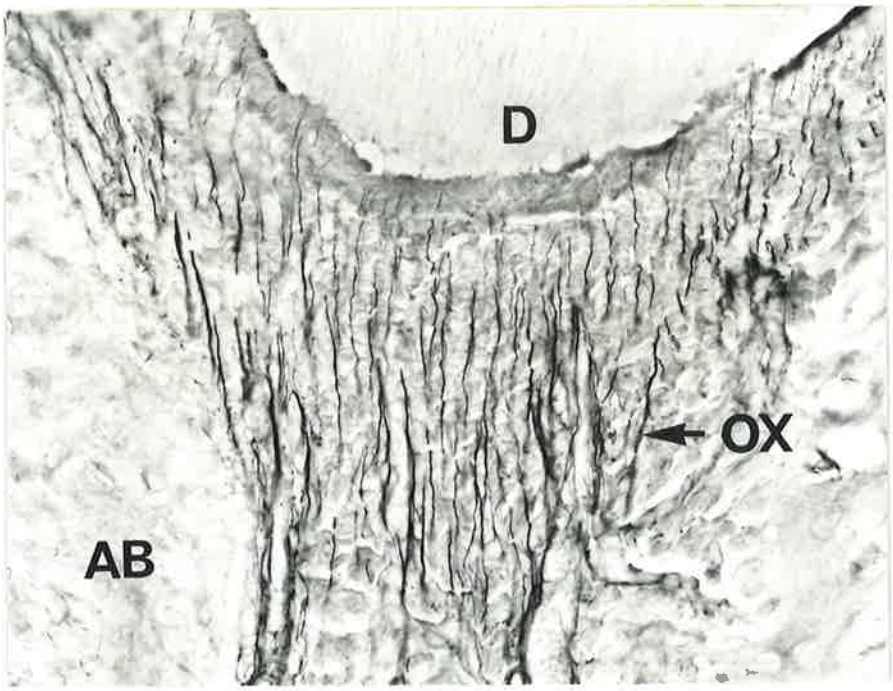

Figure 4.10 Oxytalan fibres in the cervical region of an intruded tooth. Normal mouse. Buccal tangential section. AB: alveolar bone; D: dentine; OX: oxytalan fibres.
Oxone, aldehyde fuchsin, light green. X 450

CHAPTER V

THE OXYTALAN FIBRE IN THE PERIODONTAL LIGAMENT OF THE HUMAN MAXILLARY AND MANDIBULAR PREMOLAR

It is stated (Fullmer 1960c, 1967b) that the arrangement and distribution of oxytalan fibres in the periodontal ligament of man and mouse are similar. The oxytalan fibre pattern as seen in midsagittal sections of the periodontal ligament investing the mandibular molars of mice has been shown to possess distinct regional patterns of distribution (Sims 1973). However, in certain respects, the mouse oxytalan fibre system differs from the arrangement described by previous investigators in other species. For example, the mouse oxytalan system forms a dense three-dimensional meshwork which extends between the dentinocemental junction to the vessels in the periodontal ligament. Moreover, in the mouse the oxytalan fibres do not insert into the alveolar bone (Sims 1973, 1975) as reported in humans (Fullmer 1961, 1966a, 1967b; Goggins 1966).

This investigation was undertaken to compare the arrangement of the human oxytalan fibre system on the buccal aspect of premolar teeth with the oxytalan fibre arrangement patterns in the mouse periodontal ligament.

MATERIALS AND METHODS

Specimens of human periodontal ligament were obtained from six patients aged between ten and fourteen years who required the extraction of premolars prior to orthodontic treatment. Maxillary and

mandibular premolars were surgically removed under local anaesthesia with xylocaine containing 2% lignocaine hydrochloride and adrenaline 1:80,000 (Astra, Sydney, Australia). A slender segment of buccal bone extending from the alveolar crest to the apical region was isolated from the surrounding periodontal structures and left attached to each extracted premolar. The teeth and their tissues were fixed in Lillie's (1965) aqueous neutral calcium acetate formalin and decalcified in 5% nitric acid until x-ray examination revealed that demineralization was complete. Tissue blocks were then washed in running water for eight hours, neutralized, and processed by the double embedding method for histological examination (Appendix I). Serial sections were cut at 8 μm through the periodontal ligament in the buccolingual and mesiodistal planes. Every fifth section was oxidized with a 10% aqueous solution of oxone for 1 h at 37°C, and stained with a modified version of the Fullmer et al. (1974) aldehyde fuchsin procedure, or the orcein-Halmi method (Fullmer 1959a). Adjacent control sections were stained by the same techniques omitting prior oxidation. Some sections were counterstained with haematoxylin and eosin to provide additional information on the relation of oxytalan fibres to the vascular structures (Appendix II).

FINDINGS

Buccolingual sections of human maxillary and mandibular premolars showed that the oxytalan fibres formed a three-dimensional branching meshwork which invested the root. Oxytalan fibres extended from the dentinocemental junction to the blood vessels in the periodontal ligament (Figure 5.1). Within the cementum the oxytalan fibres were of fine dimensions. As they emerged from the cementum,

generally at right angles to its surface, the fibres contributed to a delicate plexus investing the root. From the plexus the fibres curved toward more apically located vessels with which they became associated. Many of the oxytalan fibres markedly increased in thickness as they assumed an occluso-apical orientation. Maximal fibre thickness occurred amongst the vertically orientated fibres located opposite the middle and apical portions of the root.

At the cervical region some oxytalan fibres extended horizontally above the alveolar crest to disappear where they approximated the gingival elastic fibres. Oxytalan fibres emerging from the cementum just below the cementoenamel junction curved upward to associate with the vessels adjacent to the basement membrane of the gingival epithelium.

A characteristic anastomosing vascular system was present in the buccal aspect of the periodontal ligament from the cervical to the apical regions. This vascular plexus extended to the subepithelial region and was associated with a complex arrangement of oxytalan fibres (Figure 5.2).

At low magnification many of the thicker and more predominant oxytalan fibres appeared to be single strands (Figure 5.3). However, high magnification revealed that these fibres, which had a preferred orientation, were not single strands but were complex branching structures continually dividing and reuniting to form a fine inter-communicating arrangement between the other adjacent fibres (Figure 5.4). This oxytalan fibre arrangement resulted in a dense meshwork which was interwoven between the collagen fibres and formed a separate three-dimensional fibre system within the periodontal ligament. Mesiodistal sections cut tangential to the buccal group of periodontal

fibres revealed the larger oxytalan elements arranged predominantly in a palisade pattern orientated in an occluso-apical direction. In some regions the thicker fibres were aggregated to form oxytalan fibre tracts which linked the vessels vertically (Figure 5.3).

The oxytalan fibres exhibited two types of vascular association in the human periodontal ligament. Some fibres were randomly associated with individual vessels without demonstrating preferential attachment to any particular vascular type. The second arrangement revealed the presence of unique oxytalan-vascular structures. In these structures the fibres were associated not only with the walls of individual arteries, veins, and lymph vessels but the oxytalan meshwork also surrounded the total vessel complex (Figure 5.5).

DISCUSSION

This study has confirmed similarities in the arrangement of the oxytalan fibre systems in the periodontal ligament of man and that of the mouse described in Chapter II. In man the three-dimensional oxytalan meshwork comprised a substantial structure within the periodontal ligament. The coronal, middle and apical portions of the ligament revealed an oxytalan fibre arrangement analogous to that present in mouse molars in the arbitrarily defined regions B, C and E (Sims 1973). Failure to appreciate the presence of a meshwork arrangement has resulted in reports that thick apico-occlusally aligned oxytalan fibres observed in individual sections have no attachment within the periodontal ligament (Fullmer 1961, 1966b; Goggins 1966; Edwards 1968).

Although the human and mouse tissues showed the same general

features, minor differences were present. Oxytalan fibres in the human meshwork were comparatively fine, very numerous and possessed a somewhat more complex branching arrangement. Muscle attachments near the gingival margin were absent. A modification in the oxytalan meshwork corresponding with a fulcrum region was not observed.

No evidence was found to support the claims that the fibres are inserted into the alveolar bone (Fullmer 1961, 1966a, 1967b; Rannie 1963; Goggins 1966) or that they arise from it (Fullmer 1967b). Contrary to previous observations by Fullmer (1967b) oxytalan fibres did not unite the tooth to bone at the apex and generally branched prior to their association with vessels. The absence of oxytalan fibre insertion in human alveolar bone (Sims 1975, 1976) confirmed a similar finding in the mouse (Sims 1973). Fullmer et al. (1974) generally endorse this observation stating that oxytalan fibres rarely insert into bone, thus revising the previously held concept of an oxytalan fibre-alveolar bone association.

An additional finding was that the delicate oxytalan fibre plexus ensheathing the root surface was embedded in the cementum of man and mouse. This last-mentioned finding differed from Goggins' (1966) conclusion in the human.

The predominant occluso-apical orientation of the largest fibre components of the oxytalan system resulted in a palisade arrangement paralleling the pattern of the principal vessels in the periodontium of the rat molar as described by Kindlova and Matena (1962), the venous network of the human alveolus reported by Castelli (1962), vessels in the premolar region of the human periodontal ligament (Sims 1976), and the vascular supply of the mouse molar illustrated by Sims (1977b). Moreover, the oxytalan meshwork of the

cervical buccal plexus of man and mouse showed a similar orientation to the vessels in the marginal periodontium of Macacus rhesus (Kindlova 1965) and rats (Kindlova 1968).

Where oxytalan fibre tracts were sectioned transversely to their principal orientation they demonstrated their multivascular relationship with groups of vessels of various types. The two types of oxytalan-vascular arrangement in the human were identical with those found in the mouse (Figures 2.20 and 5.5). Thus, the oxytalan fibre meshwork provided a system which linked the vessels with the teeth and gingivae in man as in the mouse. Even though Fullmer et al. (1974) state that the distribution of oxytalan fibres in the periodontal ligament of man is tooth orientated, the findings of the present author in man and mouse emphasize the viewpoint that anatomically the oxytalan fibre meshwork could equally be considered to be vessel orientated.

The significance of the oxytalan component and its morphological arrangement upon the function of the periodontal membrane is not determined. However, a scissors-like behaviour of the three-dimensional oxytalan meshwork could provide an effective means of vascular response to variations in the phase and direction of functional force application to the tooth crown. In these circumstances alterations in the geometry of the oxytalan meshwork, as a result of tooth oscillation, would parallel the behaviour of the collagenous meshworks in skin as described by Viidik (1973).

SUMMARY

1. The buccal periodontium of human premolars had a similar oxytalan fibre system to that of mouse molars.
2. Oxytalan fibres displayed two types of vascular association. One

arrangement was a generalized relationship with individual periodontal vessels of all types. The second type of association involved the formation of complex oxytalan-vascular units.

3. Oxytalan fibres did not insert into human alveolar bone.

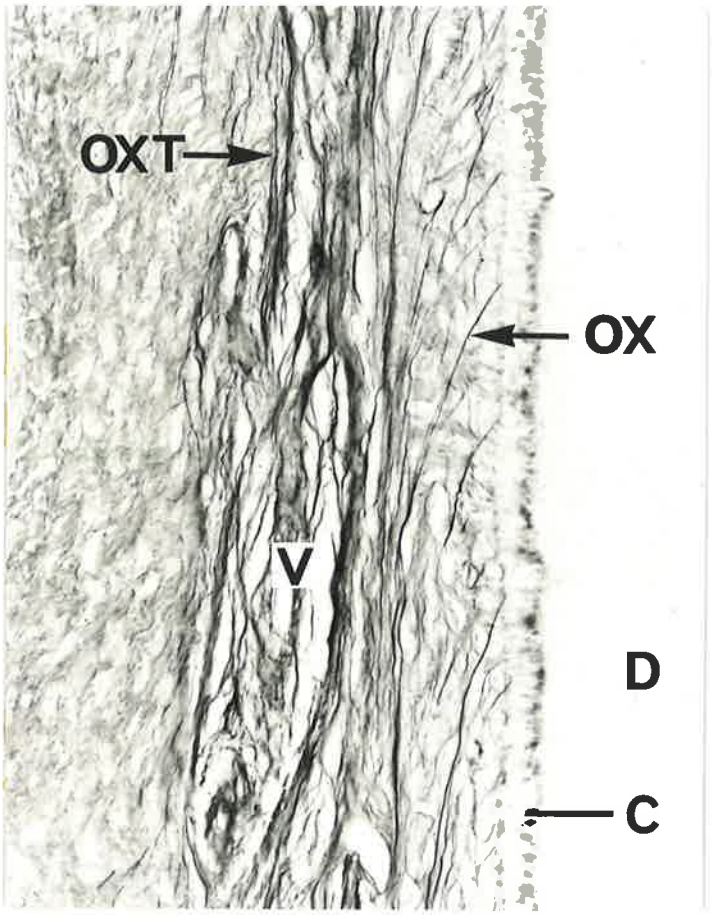

Figure 5.1 Oxytalan fibre system in a mesiodistal section through the buccal periodontal ligament of a maxillary premolar. C: cementum; D: dentine; OX: oxytalan fibre; OXT: oxytalan fibre tract; V: vessels.
Oxone, aldehyde fuchsin, light green. X 250

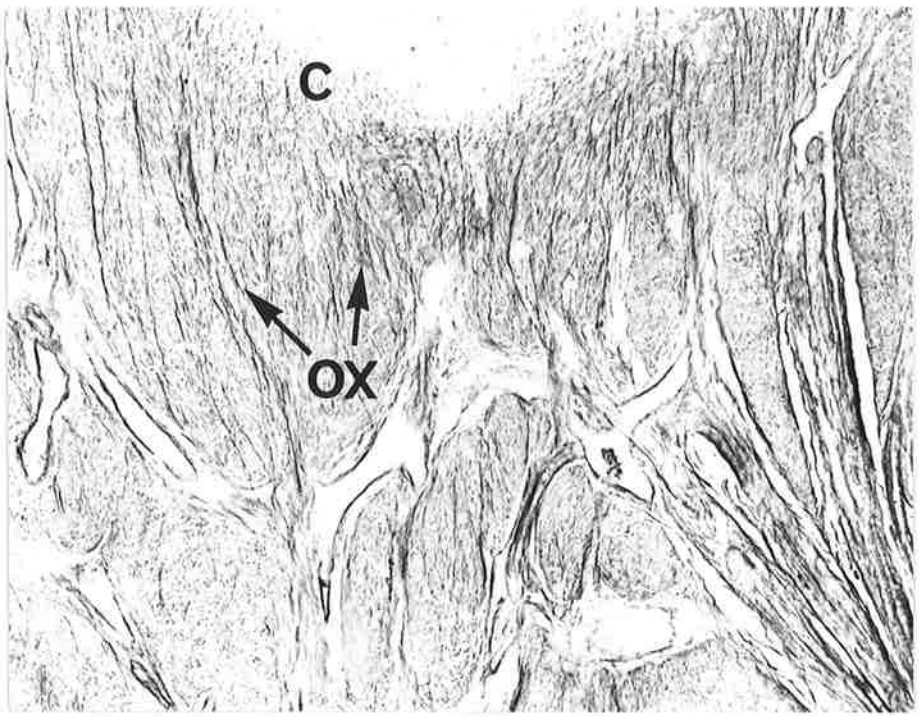

Figure 5.2 Vascular plexus in the buccal periodontal ligament of a maxillary premolar. The oxytalan fibre meshwork extends from the cementum to the vessels. Mesiodistal section, cervical region. C: cementum; OX: oxytalan fibres.
Oxone, aldehyde fuchsin, light green. X 110

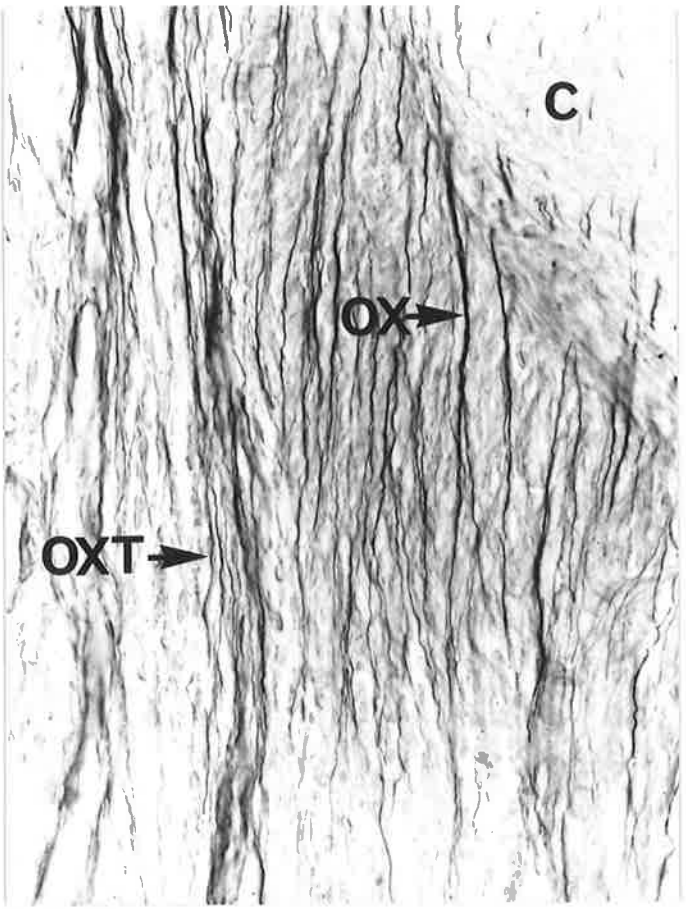

Figure 5.3

Oxytalan fibre meshwork. Mesiodistal section buccal to a maxillary premolar. C: cementum; OX: oxytalan fibre; OXT: oxytalan fibre tract. Oxone, aldehyde fuchsin, light green. X 352

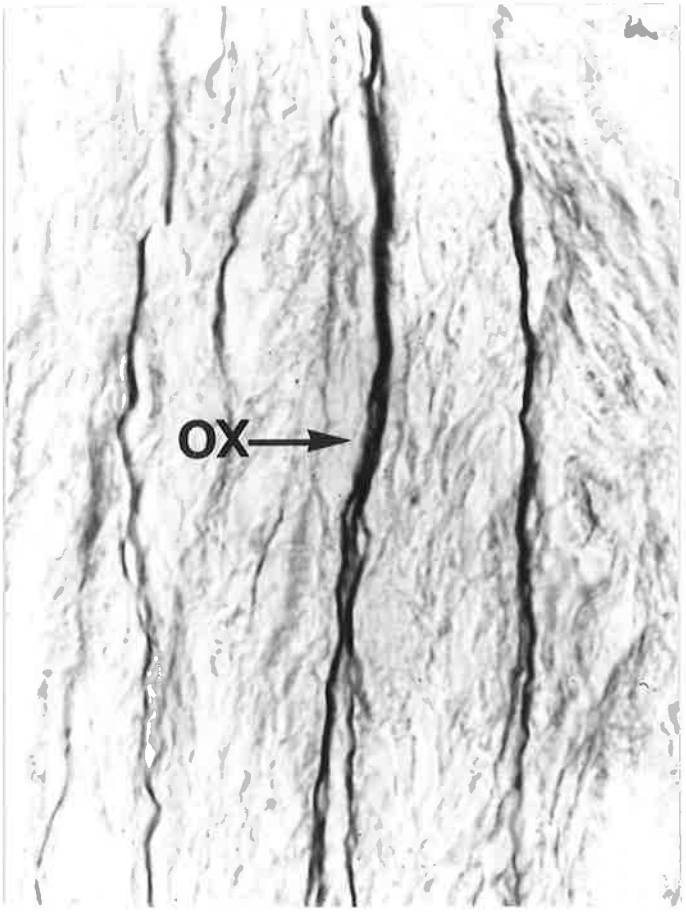

Figure 5.4

Oxytalan fibres in Figure 5.3 magnified to show branching. X 1371

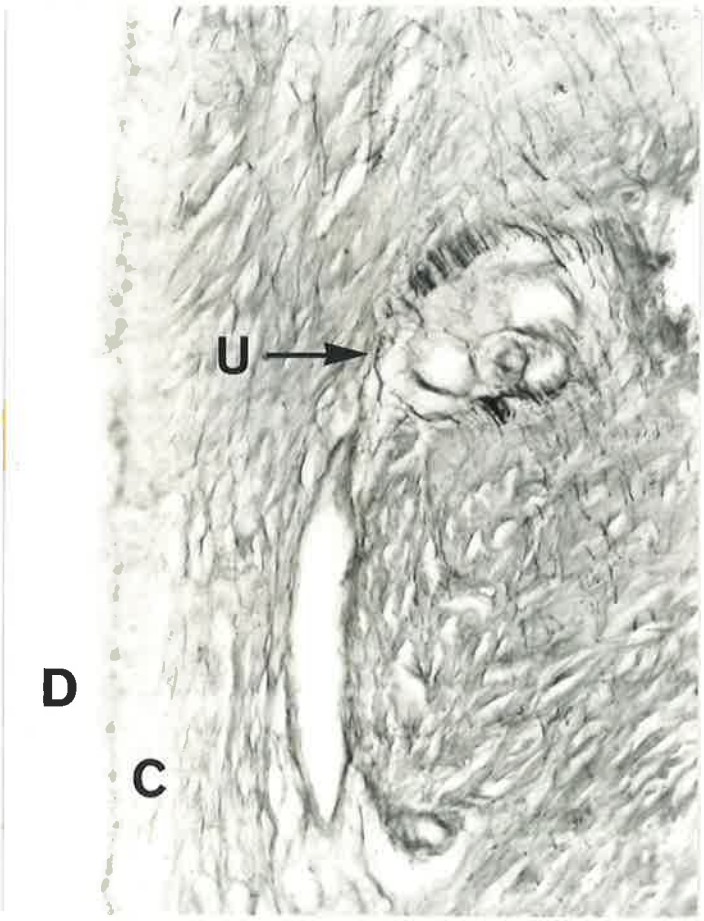

Figure 5.5 Oxytalan-vascular unit on the palatal side of a maxillary premolar. Oxytalan fibres are present in the walls of individual vessels and also circumscribe the vascular complex. C: cementum; D: dentine; U: oxytalan-vascular unit. Oxone, aldehyde fuchsin, light green. X 352

CHAPTER VI

GENERAL DISCUSSION

Mastication is one of the many complex and essential physiological functions performed by the stomatognathic system. This function requires the highly coordinated interaction of a variety of different tissues including muscles, bones, teeth, the periodontal structures and their related neural systems (Kawamura 1974).

In recent years the relationship between the structure and functional characteristics of the periodontal ligament has received increasing recognition. The identification of the oxytalan fibre in the periodontal ligament of man and animals (Fullmer 1958; Fullmer and Lillie 1958) has added a further component to be evaluated for its contribution to connective tissue biomechanics.

Nearly twenty years have passed since the oxytalan fibre was first discovered (Fullmer 1958; Fullmer and Lillie 1958). However, there remains a lack of conclusive information as to the mechanism of the oxidation procedures (Fullmer and Lillie 1958; Fullmer 1960; Mander et al. 1968; Fullmer et al. 1974), the staining reactions used for its identification (Bangle 1954; Fullmer and Lillie 1958; Fullmer 1960b;Rannie 1963; Mander et al. 1968; Fullmer et al. 1974), and the biochemical nature of the fibre itself (Fullmer and Lillie 1958; Fullmer 1959a, 1959b, 1960b, 1960c, 1967b; Fullmer et al. 1974). Consequently, it was interesting to observe in the lathyritic periodontal ligament that the oxytalan fibre retained its characteristic requirement for oxidation prior to staining with aldehyde fuchsin and orcein. This

finding indicated that the properties of the oxytalan fibre associated with oxidation and staining were not readily altered by severe pathological conditions (Tedeschi and Sommers 1961; Fisher and Fullmer 1962; Fullmer and Witte 1962).

Although the oxytalan fibre meshwork is now known to form a substantial and well-organized system within the periodontium of mouse and man, the precise function of this meshwork remains undetermined and the subject of varying opinions. Nevertheless, a review of the findings of other investigators and those of the present author provides information which lends itself to some interesting speculation. Fullmer (1958, 1967b), Fullmer and Lillie (1958) and Fullmer et al. (1974) emphasize that oxytalan fibres occur in areas of connective tissue subject to stress such as the periodontal ligament, tendons, and the adventitia of blood vessels. On numerous occasions attention has been drawn to the association of the fibres with the vascular system (Fullmer 1958, 1959b, 1961, 1964, 1967b; Kohl and Zander 1962; Rannie 1963; Carmichael 1968; Sheetz et al. 1973; Sims 1973, 1975, 1976, 1977b; Fullmer et al. 1974). And it is often overlooked that even in tendons there is a rich blood supply (Schatzker and Branemark).

Rannie (1963) proposes that the oxytalan fibres may prevent ischaemia due to obliteration of vascular channels. Simpson (1967) considers oxytalan fibres to be anchoring fibres which reinforce the periodontal ligament. Carmichael (1968) believes that the fibres are concerned with the possible stability and patency of periodontal vessels under functional pressure. However, in the present investigations the principal oxytalan fibres did not appear to be associated with the walls of the vessels in a manner that would provide mechanical support to maintain their patency during masticatory pressure. Furthermore, the observation that oxytalan fibres persisted

when they accompanied vessels traversing the interseptal crest furnished evidence which contradicted Carmichael's (1968) hypothesis since these vessels would be protected from occlusion of their lumen, even though alveolar bone is known to undergo functional distortion (Picton 1965).

Lathyrism reduces the tensile strength of the periodontal ligament (Levene and Gross 1959; Sciaky and Ungar 1961; Sarnat and Sciaky 1965). Yet, in this pathological condition where a compensatory mechanism to increase mechanical strength would have been beneficial, no proliferation of the oxytalan fibre system occured (Fullmer 1959b; Fullmer et al. 1974). The absence of oxytalan fibre proliferation in these circumstances seemed to provide evidence against the concept that the oxytalan meshwork adds mechanical strength to the periodontal ligaments and also prevents vascular obliteration. Furthermore, vascular obliteration was not observed in the periodontal ligament in lathyritic animals unless intrusive springs were applied to teeth when only isolated regions of vascular occlusion resulted at the extreme apex of the roots. Of course, lack of oxytalan fibre hypertrophy and obliteration might be explained by lathyritic interference with oxytalan fibre formation which some authors believe to occur with the oxytalan analogue, elastin, during lathyrism (Tanzer 1965).

Löe and Nuki (1964) propose that oxytalan fibres are neural elements because of staining and morphological similarities. This opinion is refuted by Goggins (1966) and the present author. Boese (1969), Edwards (1968, 1970, 1971) and Campbell et al. (1975) suggest that oxytalan fibres contribute to orthodontic relapse. However, the basis for this concept is unfounded since it has been demonstrated (Sims 1976) that during orthodontic tooth movement in man reconstitution of the oxytalan meshwork occurs in which the formation of new oxytalan fibres appears to be more rapid than oxytalan fibre degradation.

Beertsen and his colleagues (1974) surmise that oxytalan fibres function as a guiding and supporting system for the eruption of the roden incisor. This idea is also open to question since studies by the present author reveal that the oxytalan fibre system persists in the erupted molars and continually erupting incisors of old mice. This persistence is not inconsistent with an eruptive role for the fibres.

On the basis of the unique orientation of the mouse oxytalan meshwork revealed in the static histologic image Sims (1973) attributed a different function to the oxytalan fibre system. Because of the close relationship of the oxytalan fibre meshwork to the vascular walls and the extension of the oxytalan meshwork between the dentinocemental junction and the vessel walls, Sims hypothesized that under varying states of tension the oxytalan meshwork could participate in a form of mechanism, sensory or otherwise, that contributed to the regulation of vascular flow according to different functional tooth loads (Figure 6.1). This concept that the oxytalan meshwork formed a type of proprioceptive system between the oscillating tooth and the periodontal vasculature was related to the theories of Bien (1966) who described a vascular system involved in the hydrodynamic damping of tooth movement.

Sims (1975) reported that both the human and mouse periodontal ligaments contained similar, dense, three-dimensional oxytalan meshworks which, apical to the alveolar crest, exhibited a predominantly occluso-apical orientation with a lateral intercommunicating system of fine branching fibrils. It was suggested that this three-dimensional meshwork could exhibit a scissors-like behaviour providing an effective means for vascular reponse to variations in the phase and direction of functional force application to the tooth crown. In these circumstances, alterations in the geometry

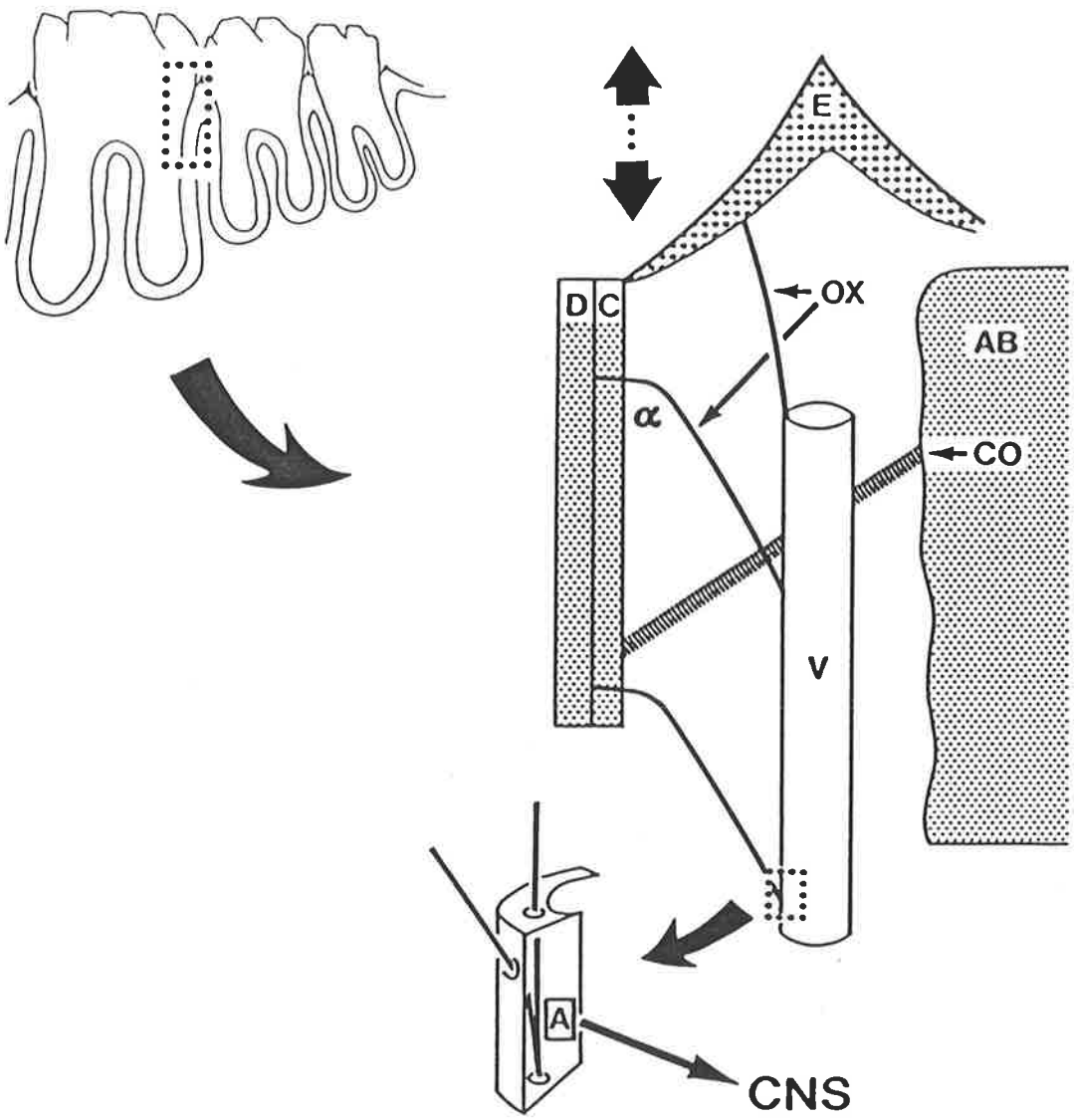

Figure 6.1 Diagrammatic representation of the functional proprioceptor hypothesis. Loading and unloading of the tooth and gingival tissues produce tensional changes in vessel walls via the oxytalan fibre system. Tensional variations adjacent to nerve axons could result in transduction of a mechanical stimulus to a neural response relayed to the central nervous system, thus influencing reflex vasomotor control. With changes in masticatory load it is also proposed that there are changes in the angular attachment (α) of oxytalan fibres which function independently from the collagen fibre system. Oxytalan and collagen fibres are proposed to undergo contrasting tensional changes during periodontal loading.
A: axon group; AB: alveolar bone; C: cementum;
CNS: input to central nervous system; CO: collagen fibre;
D: dentine; E: epithelium; OX: oxytalan fibres

of the oxytalan meshwork as a result of tooth oscillation would parallel the behaviour of collagenous and other fibre meshworks described by Viidik (1973).

Histological observations suggested that oxytalan fibre possesses elastomeric properties. Oblique sections through the mouse periodontal ligament demonstrated large oxytalan fibres with turned over ends similar to the comma shapes noted by Rannie (1963) and Goggins (1966). Oxytalan fibre stretching, recoil, and waviness due to relaxation was recorded in the molar intrusion-extrusion studies. Further evidence of oxytalan fibre recoil and elasticity was also seen in the interradicular regions where drill holes made in the living tissues for the insertion of extrusion springs resulted in waviness of the major fibres at the site of severance. These findings lend support to the view (Sims 1976) that the oxytalan fibre meshwork is composed of an elastomeric material which is normally in a state of prestress since other tissues of the body are known to exist in a state of prestress (Harkness 1968; Tkaczuk 1968; Crisp 1972).

In view of the possible elastomeric nature of the oxytalan fibre, Sims (1976) reviewed some concepts of elastin-collagen interaction in connective tissues suggesting that such information might indicate possible modes of oxytalan-collagen operation in the periodontal ligament. The function and behaviour of any particular connective tissue is affected by the type and arrangement of the component meshwork fibres, their size and rate of loading, and also the inherent properties of the fibres themselves (Harkness 1968; Viidik 1973). Therefore, it was inferred that the properties of the oxytalan fibre are probably complementary to those of collagen (Piez, Miller and Martin 1964) and quite significant in the special functional requirements of the periodontal ligament. Accordingly, Sims (1976) proposed that

oxytalan-collagen interaction could parallel the role of elastin-collagen reciprocity in which elastin is stated to be the major stress-bearing component in the low strain region for connective tissue structures such as ligament, skin and artery (Ross 1973).

Pertinent to this concept is the suggestion of Barbenel, Evans and Finlay (1973) that the major differences in the behaviour of tissues appear as differences in the initial low load extension. Should this proposal be correct it seems unlikely that oxytalan fibre function is explained on the basis of providing a significant increase in the mechanical strength of the periodontal ligament (Carmichael 1968; Rannie 1963; Simpson 1967), or maintaining vascular patency (Rannie 1963; Carmichael 1968).

Where fibre tracts were sectioned transversely to their principal orientation they showed their multivascular relationship with groups of vessels of various types. Kadar, Veress and Jellinek (1969) have suggested that pulsation within arteries provides the stimulus for the formation of elastic fibres. Although elastic and oxytalan fibres appear to be closely related (Fullmer 1966a, 1967b), Carmichael and Fullmer 1966; Greenlee et al. 1966; Sheetz et al. 1973; Fullmer et al. 1974), the presence of a pulse wave within the periodontium, reviewed by Picton (1969), could possibly account for the association of the oxytalan meshwork with the different types of periodontal vessels. However, it seems unlikely that pulsation of periodontal arteries is necessarily the sole stimulus to oxytalan meshwork formation.

The periodontal ligament is known to contain a variety of receptors (Kawamura 1971) which respond to different types of mechanical stimuli (Catton 1970). While some periodontal mechanoreceptors show slow adaptation (Hilton 1971), there are also fast adapting mechanoreceptors which respond to phasic tooth behaviour (Catton 1970).

It has been hypothesized that the oxytalan system may constitute a receptor mechanism which regulates vascular flow according to functional tooth movements (Sims 1973). As an extension of this concept it has been postulated (Sims 1975) that the oxytalan vascular association could contribute, as a result of phasic tooth behaviour, to a mechano-electrical form of coupling (Loewenstein and Rathkamp 1958; Johansson 1971) in the periodontal vessel walls.

Recent information on the oxytalan fibre system (Sims 1976, 1977b, 1977c) has led the author to extend his earlier hypothesis and suggest that the oxytalan fibre meshwork forms part of a stretch-sensitive mechanism which registers multidirectional tooth oscillation and gingival distortion as tensional variations in the walls of vessels in the periodontal ligament. Mechanical coupling between individual mouse molars is routinely provided by extension of the oxytalan meshwork across the interseptal bone. Consequently, the meshwork is believed to provide a stretch-sensitive periodontal mechanoreceptor system, additional to those already identified (Kawamura 1974), which extends throughout the periodontal ligaments with the result that stimulation of one tooth results in stimulation of the oxytalan meshworks of adjacent teeth. Comparable receptor fields are described for other mechanosensitive units in the periodontal ligaments of approximating teeth (Hannam 1970).

The author's original hypothesis also required that the oxytalan fibre system should be capable of independent function within the periodontal ligament. Although the acquisition of meaningful data from the comparatively small periodontal ligament is technically difficult to obtain (Fullmer 1967b), experimental tooth movement furnished both histological and statistical evidence in support of independent oxytalan fibre function.

Current knowledge indicates that the oxytalan fibre meshwork is a unique structure which demonstrates many similarities in the periodontal ligament of mouse and man (Fullmer 1958, 1959b, 1960b, 1960c, 1961, 1962, 1965b, 1967b; Fullmer and Lillie 1958; Fullmer et al. 1974; Sims 1973, 1975, 1976). Because of these similarities it is believed that the function of the oxytalan fibre system is comparable in both species. As Zweifach (1972) has pointed out, the biologic materials and principles which underlie the behaviour of key physiologic systems are surprisingly similar in most species.

Melcher and Walker (1976) emphasize in their recent review that our knowledge of the behaviour of the constituents of the periodontal ligament following loading is meagre. The four investigations described in this thesis demonstrate that the use of the light microscope to examine and record static histological images can provide new information on the anatomy, metabolism, and function of the oxytalan fibre in the periodontium.

Without additional experimental or other tests it would be unreasonable to be adamant about any firm functional interpretation. Of the possibilities considered by the author, the concept of a proprioceptor function appears to be the most tenable. This hypothesis, although lacking in scientific validity, forms the foundation and stimulation for other investigators to conduct further research on the subject of the oxytalan fibre.

The aims and objectives of this investigation have been fulfilled. An animal model of the oxytalan fibre meshwork has been established and the fibre shown to be metabolically and structurally

distinct from collagen. Experimental tooth movement has provided statistically significant evidence of functional changes at the site of cemental oxytalan fibre attachment. Moreover, comparison of the oxytalan fibre systems in mouse and man demonstrate corresponding vascular and osseous relationships.

Further studies are in progress to obtain additional information on the interaction between the oxytalan fibre meshwork and the vessels of the periodontal ligament. Several important questions remain to be answered. These include the biochemical structure of the protein oxytalan, its physical characteristics, the ultrastructural innervation of oxytalan fibres, a comparison of the electronmicroscopic anatomy of elastic and oxytalan components and a more definitive physiological function of the oxytalan fibre meshwork.

CHAPTER VII

APPENDICES

APPENDIX I

HISTOLOGICAL PREPARATION OF SPECIMENS

FIXATION

Tissues were immersed in either of the following solutions for 24-72 h at 25°C. The fixative was changed daily.

Neutral buffered formalin (Lillie 1965)

formalin solution (37-40%)	100.0 ml
distilled water	900.0 ml
sodium dihydrogen phosphate	4.0 g
sodium hydrogen orthophosphate	6.5 g

Calcium acetate formalin (Lillie 1965)

formalin solution (37-40%)	100.0 ml
distilled water	900.0 ml
calcium acetate (monohydrate)	20.0 g

DEMINERALIZATION

Three different methods were used. Solutions were maintained at 25°C and changed daily until radiographs indicated the completion of demineralization.

Formic/formate solution

formic acid	40.0 ml
distilled water	100.0 ml
sodium formate	7.0 g

Specimens were washed in running water for 2 h after completion of treatment.

Decal

A commercial solution prepared by Omega Chemical Corporation, New York, U.S.A. The composition is not given by the manufacturer. Demineralization of mouse mandibles occured in 3-4 h after which the tissues were washed in running water for 3 h.

The preservation of tissue morphology and staining reactions were not considered to be as satisfactory with this method as with formic/formate.

Nitric acid solution (Luna 1968)

nitric acid, concentrated (68-70% sp. gr. 1.41) ..	5.0 ml
distilled water	95.0 ml

Human tissues were treated with this solution. After demineralization the specimens were washed in running water for 3 h.

NEUTRALIZATION

Specimens were placed in 5% sodium sulphate for 12-36 h according to their size and then washed for 1-3 h.

DOUBLE EMBEDDING METHOD

Processing of specimens was carried out at 37^{o}C in the following sequence:

(1)	70% alcohol	one hour
(2)	80% alcohol	one hour
(3)	90% alcohol	one hour
(4)	95% alcohol	one hour
(5)	absolute alcohol I	one hour
(6)	absolute alcohol II	one hour
(7)	absolute alcohol III	one hour

(8)	one part absolute alcohol and	
(9)	one part methyl salicylate	one hour
(10)	methyl salicylate plus 0.5% celloidin	two days
(11)	methyl salicylate plus 1.0% celloidin	two days

Paraffin embedding

(1)	two thirds methyl salicylate and	
	one third paraffin wax	one hour
(2)	one half methyl salicylate and	
	one half paraffin wax	one hour
(3)	one third methyl salicylate and	
	two thirds paraffin wax	one hour
(4)	paraffin wax (first change)	two hours
(5)	paraffin wax (second change)	two hours
(6)	paraffin wax (third change)	overnight

Vacuuming

Specimens were vacuumed at 56^{o}C in clean paraffin wax for 15-20 min (depending on size and type of tissue).

Blocking

Tissues were blocked in clean 56^{o}C wax using a Tissue Tek II Tissue Embedding Centre and "Peelaway" moulds.

APPENDIX II

GOMORI'S ALDEHYDE FUCHSIN (Lillie 1965)

Preparation

(1) Dissolve 0.5 g crystalline basic fuchsin (C.I. 42510) in 100 ml 70% alcohol.

(2) Add 1.0 ml concentrated hydrochloric acid.

(3) Add 1.0 ml fresh U.S.P. paraldehyde.

Age at room temperature for 72-84 h until the mixture assumes a deep purple or violet colour and is ready to use. Stain must then be stored at 0-5°C and discarded after one to two weeks (Fullmer 1960a; Fullmer et al. 1974).

Note: Fixatives containing chromates or mercury must be avoided.

References

Gomori, G. 1950. Am. J. Clin. Path. 20:665.

Lillie, R.D. 1965. Histopathologic Technique and Practical Histochemistry. McGraw-Hill Book Company. New York, 3rd ed. p. 556.

Pearse, A.G.E. 1968. Histochemistry. Theoretical and Applied. J. and A. Churchill Ltd. London, 3rd ed. Vol. I, p. 639.

ALDEHYDE FUCHSIN (modified after Fullmer and Lillie 1958; Fullmer et al. 1974)

The following procedures were used to demonstrate oxytalan fibres.

Method

(1) Bring sections to water.

(a) deparaffinize in xylol for 10 min.

(b) rinse in two changes of 100% alcohol (1 min each).

(c) rinse in 70% alcohol (1 min). If the sections appear cloudy renew ALL solutions and repeat.

(d) wash in tap water.

(e) rinse in distilled water.

(2) Oxidize in a fresh 10% aqueous solution of Oxone for 30 min at 37°C.*

(3) Wash in running tap water for 2 min.

(4) Rinse in distilled water.

(5) Rinse in 70% alcohol.

(6) Stain in Gomori's aldehyde fuchsin for 8 min.**

(7) Differentiate in four changes of 95% alcohol until all excess stain is removed. (First change 1 min and three remaining changes 2 min each).***

(8) If necessary, rinse for 2 min in 4% hydrochloric acid in 70% alcohol to reduce background staining.$^{\phi,+}$

(9) Counterstain for 5-10 s in a solution of light green SF made up as follows:$^{++}$

distilled water	100.0 ml
light green (G.T. Gurr, C.I. 42095) ..	0.02 g
glacial acetic acid	1.0 ml

(10) Rinse briefly in 0.2% glacial acetic acid in 95% alcohol.

(11) Dehydrate in 100% alcohol (at least 2 changes).

(12) Clear sections in xylol and mount.

Modifications

* Fullmer et al. (1974) oxidized at 25°C.

** Fullmer and Lillie (1958) advised 8 min whereas Fullmer et al. (1974) suggested 10-20 min.

*** Fullmer et al. (1974) used different rinsing times and included a final differentiation bath of 70% alcohol for 4 min.

ϕ Löe and Nuki (1964).

\+ Lillie-Mayer haematoxylin omitted.

++ Fullmer and Lillie (1958), Fullmer et al. (1974) used a modified Halmi counterstain.

Results

Oxytalan fibres	deep violet to purple
Elastic fibres	deep violet to purple in unoxidized and oxidized sections
Basement membrane	purple
Collagen, dentine, cementum bone and epithelium	take up light green with differing shades of intensity

TAENZER-UNNA ACID ORCEIN (Lillie 1965)

Preparation

(1) Dissolve 1.0 g of orcein (natural or synthetic, G.T. Gurr) in 100.0 ml of 75% alcohol.

(2) Add 1.0 ml of concentrated hydrochloric acid.

Modification

Incubate solution for 1 h at 60°C.

Reference

Lillie, R.D. 1965.

ORCEIN-HALMI (Modified from Fullmer 1959a)

Method

(1) Bring sections to water.*

(2) Oxidize in a fresh 10% aqueous solution of Oxone for 30 min at 37°C.**

(3) Wash in running water for 2 min.

(4) Stain in orcein at 50°C for 1-8 h.***

(5) Differentiate in 70% alcohol for 5 min using 3 changes.

(6) Sections to water.****

(7) Counterstain with modified Halmi for 20 s.

(8) Rinse briefly in 0.2 acetic acid in 95% alcohol.

(9) Dehydrate and mount.

Modifications

* Fullmer brought the sections to absolute alcohol.

** Fullmer used peracetic acid.

*** Orcein staining by Fullmer was 15 min at 37°C.

**** Lillie-Mayer alum haematoxylin omitted.

Results

Oxytalan and elastic fibres	brown, brownish purple
Keratin, Dentinal tubules	brown
Bone, dentine	medium yellowish green
Collagen, epithelial cells	light yellowish green

WEIGERT'S RESORCIN FUCHSIN (Lillie 1965)

Preparation

(1) Prepare 200.0 ml of 1% basic fuchsin solution.

(2) Add 4.0 g resorcinol and boil until dissolved.

(3) Add 25.0 ml liquor ferri chloridi (2.3M $FeCl_3$) and boil 2-5 min longer.

(4) Cool, and collect precipitate on a filter.

(5) Take up the precipitate from the filter paper and the vessel with 200.0 ml 95% alcohol (by boiling as necessary).

(6) Add 4.0 ml concentrated hydrochloric acid and filter, washing the filter through afterwards with enough fresh alcohol to restore the total volume to 200.0 ml,

OR

(7) Use commercially available product (British Drug Houses, Poole, England).

RESORCIN FUCHSIN (Modified from Luna 1968)

Method

(1) Bring sections to water.

(2) Oxidize in 10% aqueous Oxone for 30 min at 37°C.

(3) Wash in running tap water for 2 min.

(4) Rinse in distilled water.

(5) Rinse in 95% alcohol.

(6) Stain in resorcin fuchsin for 30-60 min.

(7) Rinse in three changes of 95% alcohol.

(8) Counterstain with light green SF for 5-10 s or modified Halmi for 20 s.

(9) Rinse briefly in 0.2 acetic acid in 95% alcohol.

(10) Dehydrate and mount.

Results

Oxytalan and elastic fibres ..... purple to black

ORCINOL-NEW FUCHSIN (Fullmer and Lillie 1956b)

The dye was prepared and used as detailed by Fullmer and Lillie (1956b).

VERHOEFF'S IODINE IRON HAEMATOXYLIN (Mallory)

The preparation and staining technique was according to Lillie (1965): pages 551-552.

HAEMATOXYLIN AND EOSIN Y (Lillie-Mayer

Preparation and method was that cited in Lillie (1965): pages 174, 176-177.

VAN GIESON COLLAGEN FIBRE STAIN

Prepared and used according to Lillie (1965): pages 539-540.

GORDON AND SWEET SILVER IMPREGNATION

This stain for reticulin was used as cited in Pearse (1968): pages 641-642.

Modification

The tissues were toned in gold chloride (0.5% aqueous) for 2 min.

NAOUMENKO AND FEIGIN RETICULIN STAIN

This silver stain was applied according to the published directions (1974).

Modification

The tissues were toned in 0.5% aqueous gold chloride for 2 min.

LILLIE'S ALLOCHROME CONNECTIVE TISSUE METHOD

The solutions and staining method followed the procedure in Lillie (1965): pages 549-550.

METHODS FOR NERVE FIBRES

The solutions and methods were those cited in Luna (1968).

(1) Bielschowdky: pages 193-194.

(2) Bodian: pages 195-196.

(3) Hirano-Zimmerman: pages 198-199.

PERIODIC ACID SCHIFF REACTION

The solutions, methods and results were those described by Pearse (1968): pages 198-202.

0.5% TOLUIDINE BLUE FOR METACHROMASIA

Method and solution as described by Pearse (1968): page 665.

CONGO RED METHOD (Modified from Highman 1946)

Used according to the directions of Pearse (1968): page 685.

THIOFLAVINE T METHOD (after Vassar and Culling 1959)

The technique was that cited in Pearse (1968): page 685.

ALCIAN BLUE METHOD FOR ACID MUCOPOLYSACCHARIDES (after Steedman 1950)

The method adapted by Pearse (1968): pages 672-673.

ALCIAN BLUE-PAS PROCEDURE (after Mowry 1963)

The method recommended by Pearse (1968): pages 673-674.

NINHYDRIN TEST

Method quoted in Lillie (1965): page 246.

APPENDIX III

ENZYME DIGESTIONS

β-glucuronidase

Type B-I (β-D-Glucuronide glucuronohydrolase E.C. No. 3.2.1.31) prepared from bovine liver (Sigma Chemical Company, St. Louis, United States). Contained approximately 20% buffer salts as sodium acetate, sodium citrate and EDTA (Na_2). Activity 989,800 Fishman units/g solid. Lot 65C-7390.

The acetate buffer (pH 4.5) was prepared according to Barka and Anderson (1963).

Elastase

Type III (Pancreatopeptidase E.C. No. 3.4.4.7) prepared from hog pancreas and chromatographically purified. A lyphilized water soluble powder (Sigma Chemical Company). Activity stated as 54 units/mg solid. Lot 84C-8140.

The Tris "Universal Buffer" (Sigma Chemical Company) was prepared at pH 8.8 from tables in Barka and Anderson (1963).

Pepsin

Prepared from hog stomach mucosa (E.C. No. 3.4.4.1). 1 × 60,000. 2x crystallized and lyphilized powder (Sigma Chemical Company). Contained 3,500 units/mg solid. Lot 14-C8050.

APPENDIX IV

RELATED PUBLICATIONS

Sims, M.R. 1973. Oxytalan fibre system of molars in the mouse mandible. J. dent. Res. 52, 797-802.

Sims, M.R. 1974. Oxytalan response to tooth intrusion and extrusion. I.R.C.S. (Research on: Anatomy and Human Biology) 2, 1418.

Sims, M.R. 1975. Oxytalan-vascular relationships observed in histologic examination of the periodontal ligaments of man and mouse. Archs oral Biol. 20: 713-716.

Sims, M.R. 1977a. The oxytalan fibre system in the mandibular periodontal ligament of the lathyritic mouse. J. Oral Path. In Press.

Sims, M.R. 1977b. Oxytalan meshwork associations observed in histological studies of the periodontium of the mouse mandible. Archs oral Biol. In Press.

Sims, M.R. 1977c. Oxytalan fibre response to tooth intrusion and extrusion in normal and lathyritic mice: A statistical analysis. J. periodont. Res. In Press.

BIBLIOGRAPHY

ALPER, R., PRIOR, J.T. and RUEGAMER, W.R. 1968. Histological and biochemical studies on the ground substance of the aortas of lathyritic rats. J. Atheroscler. Res. 8: 787-801.

AYER, J.P. 1964. Elastic tissue. In: *International Review of Connective Tissue Research*, (Edited by Hall, D.A.), Vol. 2, pp. 33-100, Academic Press, New York.

BANGLE, R. 1954. Gomori's paraldehyde-fuchsin stain. I. Physico-chemical and staining properties of the dye. J. Histochem. Cytochem. 2: 291-299.

BARATIERI, A. 1967a. The oxytalan connective tissue fibres in gingival hyperplasia in patients treated with sodium diphenyl-hydantoin. J. periodont. Res. 2: 106-114.

BARATIERI, A. 1967b. Observations on the peracetic acid-aldehyde fuchsin (oxytalan) positive tissues elements in diphenyl-hydantoin sodium gingival hyperplasia. J. dent. Res. 46: 1246.

BARATIERI, A. and MIANI, C.M. 1974. Histochemical study of oral mucosa lesions in tuberous sclerosis. The oxytalan connective tissue fibres distribution. J. dent. Res. 53: 734-735.

BARBENEL, J.C., EVANS, J.H. and FINLAY, J.B. 1973. Stress-strain-time relations for soft connective tissues. In: *Perspectives In Biomedical Engineering*, (Edited by Kenedi, R.M.), pp. 165-172, Macmillan Press, London.

BARKA, T. and ANDERSON, P.J. 1963. Histochemistry. Hoeber Medical Division, Harper and Row, New York.

BEERTSEN, W., EVERTS, V. and van den HOOF, A. 1974. Fine structure of fibroblasts in the periodontal ligament of the rat incisor and their possible role in tooth eruption. Archs oral Biol. 19: 1087-1098.

BERKOVITZ, B.K.B., MIGDALSKI, A. and SOLOMON, M. 1972. The effect of the lathyritic agent aminoacetonitrile on the umimpeded eruption rate in normal and root-resected rat lower incisors. Archs oral Biol. 17: 1755-1764.

BERNICK, S. 1957. Innervation of teeth and periodontium after enzymatic removal of collagenous elements. Oral Surg., Oral Med., Oral Path. 10: 323-332.

BERNICK, S. 1959. Innervation of the developing molar teeth of rats. Anat. Rec. 133: 91-103.

BEYNON, A.D. 1967. Oxytalan fibres in the developing mouse molar. J. dent. Res. 46: 1272.

BIEN, S.M. 1966. Fluid dynamic mechanisms which regulate tooth movement. In: *Advances in Oral Biology*, (Edited by Staple, P.H.). Vol. II, pp. 173-201, Academic Press, New York.

BIRN, H. 1966. The vascular supply of the periodontal membrane. J. periodont. Res. 1: 56-68.

BOESE, L.R. 1969. Increased stability of orthodontically rotated teeth following gingivectomy in Macaca nemestrina. Am. J. Orthod. 56: 273-290.

BRAIN, W.E. 1969. The effect of surgical transsection of free gingival fibres on the regression of orthodontically rotated teeth in the dog. Am. J. Orthod. 55: 50-70.

CAMPBELL, P.M., MOORE, J.W. and MATTHEWS, J.L. 1975. Orthodontically corrected midline diastemas. A histological study and surgical procedure. Am. J. Orthod. 67: 139-158.

CAMERON, D.S., JENNINGS, E.H. and RANNIE, I. 1970. Oxytalan fibres in the human tympanic membrane. J. Lar. Otol. 84: 1235-1239.

CARMICHAEL, G.G. 1968. Observations with the light microscope on the distribution and connexions of the oxytalan fibre of the lower jaw of the mouse. Archs oral Biol. 13: 765-772.

CARMICHAEL, G.G. 1969. Personal communication.

CARMICHAEL, G.G. and FULLMER, H.M. 1966. The fine structure of the oxytalan fibre. J. cell Biol. 28: 33-36.

CARMICHAEL, G.G. and OAKES, B.W. 1970. An ultrastructural study of elastic fibres in the articular tissue of the rat. Aust. Conference Electron Microscopy, p. 60, Canberra. (abstract).

CARNEIRO, J. and Fava de MORAES, F. 1965. Radioautographic visualization of collagen metabolism in the periodontal tissues of the mouse. Archs oral Biol. 10: 833-848.

CASTELLI, W. 1962. Vascular architecture of the human adult mandible. J. dent. Res. 42: 786-792.

CASTELLI, W.A. and DEMPSTER, W.T. 1965. The periodontal vasculature and its responses to experimental pressures. J. Am. dent. Assoc. 70: 890-905.

CATTON, W.T. 1970. Mechanoreceptor function. Physiol. Rev. 50: 297-318.

COTTA-PEREIRA, G., RODRIGO, F.G. and DAVID-FERREIRA, J.F. 1976. The use of tannic acid-glutaraldehyde in the study of elastic and elastic-related fibres. Stain Technol. 51: 7-11.

CRISP, J.D.C. 1972. Properties of tendon and skin. In: *Biomechanics: Its Foundations and Objectives*, (Edited by Perrone, N. and Anliker, M.), pp. 141-179, Prentice-Hall, New Jersey.

CULLING, C.F.A. 1974. Handbook of histopathological and histochemical techniques. 3rd ed., Butterworths, London.

DALY, C.H., NICHOLLS, J.I., KYDD, W.L. and NANSEN, P.D. 1973. Biomechanics of the oral tissues. In: *Perspectives in Biomedical Engineering*, (Edited by Kenedi, R.M.), pp. 181-185, Macmillan Press, London.

DASLER, W. and MILLISER, R.V. 1957. Experimental lathyrism in mice fed diets containing sweet peas or B-aminopropionitrile. Proc. Soc. exp. Biol. Med. 96: 171-174.

DAWES, J.D.K. 1970. The aetiology and sequelae of exudative otitis media. J. Lar. Otol. 84: 583-610.

DUTHY, L.M. 1972. Elastin and Oxytalan Fibres in Rabbit Joints. Master of Dental Surgery Research Project, The University of Adelaide.

EDWARDS, J.G. 1968. A study of the periodontium during orthodontic rotation of teeth. Am. J. Orthod. 54: 441-461.

EDWARDS, J.G. 1970. A surgical procedure to eliminate rotational relapse. Am. J. Orthod. 57: 35-46.

EDWARDS, J.G. 1971. The prevention of relapse in extraction cases. Am. J. Orthod. 60: 128-141.

FISHER, A.K. and FULLMER, H.M. 1962. Oxytalan fibres in ameloblastomas. Oral Surg., Oral Med., Oral Path. 15: 246-248.

FULLMER, H.M. 1958. Differential staining of connective tissue fibres in areas of stress. Science, 127: 1240.

FULLMER, H.M. 1959a. The peracetic-orcein-Halmi stain: A stain for connective tissues. Stain Technol. 34: 81-84.

FULLMER, H.M. 1959b. Observations on the development of oxytalan fibres in the periodontium of man. J. dent. Res. 38: 510-518.

FULLMER, H.M. 1960a. Observations of the development of oxytalan fibres in dental granulomas and radicular cysts. Archs path. 70: 59-67 or 73-81.

FULLMER, H.M. 1960b. Effect of peracetic acid on the enzymatic digestion of various mucopolysaccharides: reversal of the PAS staining reaction of mucin. J. Histochem. Cytochem. 8: 113-121.

FULLMER, H.M. 1960c. A comparative histochemical study of elastic, pre-elastic and oxytalan connective tissue fibres. J. Histochem. Cytochem. 8: 290-295.

FULLMER, H.M. 1961. A histochemical study of periodontal disease in the maxillary alveolar processes of 135 autopsies. J. Periodont. 32: 206-218.

FULLMER, H.M. 1962. A critique of normal connective tissues of the periodontium and some alterations with periodontal disease. J. dent. Res. 41: 223-229.

FULLMER, H.M. 1963. The oxytalan connective tissue fibre in health and disease. Ann. Histochim. 8: 51-54.

FULLMER, H.M. 1964. The use of histochemistry in oral histology. In: *Oral Histology:* Inheritance and Development, (Edited by Provenza, D.V.), pp. 497-529, J.B. Lippincott, Philadelphia.

FULLMER, H.M. 1965a. The use of histochemistry in oral pathology. In: *Oral Pathology*, (Edited by Tiecke, R.W.), pp. 749-785, McGraw-Hill, New York.

FULLMER, H.M. 1965b. The histochemistry of the connective tissues. In: *International Review of Connective Tissue Research*, (Edited by Hall, D.A.), Vol. 3, pp. 1-76, Academic Press, New York.

FULLMER, H.M. 1966a. Connective tissues and changes with certain oral diseases. In: *Environmental Variables in Oral Disease*, (Edited by Kreshover, S.J. and McClure, F.J.), pp. 171-199, American Association for the Advancement of Science, Washington, D.C.

FULLMER, H.M. 1966b. Histochemical studies of the periodontium. J. dent. Res. 45: 469-477.

FULLMER, H.M. 1967a. The development of oxytalan fibres. In: *The Mechanisms of Tooth Support*, (Edited by Anderson, D.J., Melcher, A.H. and Picton, D.C.A.), pp. 72-75, John Wright, Bristol.

FULLMER, H.M. 1967b. Connective tissue components of the periodontium. In: *Structural and Chemical Organization of the Teeth*, (Edited by Miles, A.E.W.), Vol. 2, pp. 349-414, Academic Press, New York.

FULLMER, H.M. 1969. Personal communication.

FULLMER, H.M. 1972. Personal communication.

FULLMER, H.M. and CARMICHAEL, G.G. 1966. The fine structure of the oxytalan fibre. J. cell. Biol. 28: No. 1, 33-36.

FULLMER, H.M. and LILLIE, R.D. 1956a. Some aspects of the mechanism of orcein staining. J. Histochem. Cytochem. 4: 64-68.

FULLMER, H.M. and LILLIE, R.D. 1956b. A selective stain for elastic tissue (orcinol-new fuchsin). Stain Technol. 31: 27-29.

FULLMER, H.M. and LILLIE, R.D. 1957. The staining of collagen with elastic tissue stains. J. Histochem. Cytochem. 5: 11-14.

FULLMER, H.M. and LILLIE, R.D. 1958. The oxytalan fibre: A previously undescribed connective tissue fibre. J. Histochem. Cytochem. 6, 425-430.

FULLMER, H.M., SHEETZ, J.H. and NARKATES, A.J. 1974. Oxytalan connective tissue fibres. A review. J. Oral Path. 3, 291-316.

FULLMER, H.M. and WITTE, W.E. 1962. Periodontal membrane affected by scleroderma: A histochemical study. Archs Path. 73: 184-189.

FUNG, Y.C. 1972. Stress-strain-history relations of soft tissues in simple elongation. In: *Biomechanics: Its Foundations and Objectives*, (Edited by Fung, Y.C., Perrone, N. and Anliker, M.), pp. 181-208, Prentice-Hall, New Jersey.

GARDNER, A.F. 1959. Influence of injury and adaptation of the periodontal ligament to pathologic changes during experimental lathyrism. J. Periodont. 30: 253-264.

GARDNER, A.F. 1960. Morphologic study of oral connective tissues in lathyrism. J. dent. Res. 39: 24-45.

GARDNER, A.F., DASLER, W. and WEINMANN, J.P. 1958. Masticatory apparatus of albino rats in experimental lathyrism. J. dent. Res. 37: 492-515.

GARFUNKEL, A. and SCIAKY, I. 1971. Vascularization of the periodontal tissues in the adult laboratory rat. J. dent. Res. 50, 880-887.

GAWLIK, Z. and JAROCIŃSKA, M. 1964. A new and simple method of staining oxytalan fibres. Folia Histochem. Cytochem. 2: 257-260.

GAWLIK, Z. 1965. Morphological and morphochemical properties of the elastic system in the motor organ of man. Folia Histochem. Cytochem. 3: 233-251.

GERZELI, G. and CUCCHI, M.L. 1975. Ultrastructural studies on the myocardial capillaries of the experimentally lathyritic rat. Protective effect of certain flavonoids. Virchows Arch. path. Anat. and Histol. 365: 201-212.

GILLMAN, T., PENN, J., BRONKS, D. and ROUX, M. 1955. Abnormal elastic fibres. Archs Path. 59: 733-749.

GLICK, D. 1967. Usage of "histochemical" "staining" and "biochemical" in histochemical literature. J. Histochem. Cytochem. 15: 299.

GOGGINS, J.F. 1965. The Oxytalan Fibre: Its Fine Structure and Distribution in the Deciduous Periodontal Membrane. M.S. Thesis, Marquette University, Milwaukee, quoted in Fullmer et al. 1974 Oxytalan connective tissue fibres: A review. J. Oral Path. 3: 291-316.

GOGGINS, J.F. 1966. The distribution of oxytalan connective tissue fibres in the periodontal ligaments of deciduous teeth. Periodontics 4: 182-186.

GOMORI, G. 1950. Aldehyde-fuchsin: a new stain for elastic tissue. Am. J. Clin. Path. 20: 665-666.

GREENLEE, T.K., ROSS, R. and HARTMAN, J.L. 1966. The fine structure of elastic fibres. J. cell. Biol. 30: 59-71.

GRIFFIN, C.J. and HARRIS, R. 1967. The fine structure of the developing human periodontium. Archs oral Biol. 12: 971-982.

HAM, K.N. 1962. The fine structure of rat aorta in experimental lathyrism. Austral. J. exp. Biol. 40: 353-366.

HAMNER, J.E. and FULLMER, H.M. 1966. Oxytalan fibres in benign fibro-osseous jaw lesions. Archs Path. 82: 35-39.

HANNAM, A.G. 1970. Receptor fields of periodontal mechanosensitive units in the dog. Archs oral Biol. 15, 971-978.

HARKNESS, R.D. 1968. Mechanical properties of collagenous tissues. In: *Treatise on Collagen*, (Edited by Gould, B.S.), Vol. 2, Biology of Collagen, Part A, pp. 247-310, Academic Press, London.

HARRIS, R. and GRIFFIN, C.J. 1967. The protein-polysaccharide complex of the developing human periodontium. Archs oral Biol. 12: 1107-1114.

HASEGAWA, J. 1960. "Oxytalan" fibres of the dermal-epidermal junction. Archs Dermat. 82: 250-252.

HILTON, P.B. 1971. The effect of repeated stimulation on the response from slowly adapting periodontal mechanoreceptors. In: *Oral Physiology*, (Edited by Emmelin, N. and Zotterman, Y.), Wenner-Gren Centre International Symposium Series, Vol. 20, pp. 217-222, Pergamon Press, Oxford.

HOROBIN, R.W. and JAMES, N.T. 1970. The staining of elastic fibres with direct blue 152. A general hypothesis for the staining of elastic fibres. Histochemie 22: 324-336.

HOROBIN, R.W., FLEMMING, L. and KEVILL-DAVIES, I.M. 1974. Basic fuchsin-ferric chloride: A simplification of Weigert's resorcin-fuchsin stain for elastic fibres. Stain Technol. 49: 207-210.

HURST, R.V.V. 1972. Regeneration of periodontal and transseptal fibres after autografts in Rhesus monkeys: A qualitative approach. J. dent. Res. 51: 1183-1192.

JOHANSSON, B. 1971. Electromechanical and mechanoelectrical coupling in vascular smooth muscle. Angiologica 8: 129-143.

KÁDÁR, A., VERESS, B. and JELLIŃEK, H. 1969. Relationship of elastic fibre production with smooth muscle cells and pulsation effect in large vessels. Acta Morph. Acad. Sci. Hung. 17: 187-200.

KANOUSE, M.C. 1966. Oxytalan fibres in the periodontium of ascorbic acid-deficient guinea pigs. J. dent. Res. 45: 311-314.

KAWAMURA, Y. 1971. Recent advances in the physiology of mastication. In: *Oral Physiology*, (Edited by Emmelin, N. and Zotterman, Y.), Wenner-Gren Centre International Symposium Series, Vol. 20, pp. 163-204, Pergamon Press, Oxford.

KAWAMURA, Y. 1974. Neurogenesis of mastication. In: *Frontiers of Oral Physiology*, (Edited by Kawamura, Y.), Vol. 1, Physiology of Mastication, pp. 77-120, S. Karger, Basel.

KENNEDY, G.D.C. and KENNEDY, J.S. 1963. An autoradiographic study of the dental lesions of lathyrism in the rat. Archs oral Biol. 8: 207-217.

KINDLOVA, M. 1965. The blood supply of the marginal periodontium in *Macacus rhesus*. Archs oral Biol. 10: 869-874.

KINDLOVA, M. 1967. Vascular supply of the periodontium in periodontitis. Internat. dent. J. 17: 476-489.

KINDLOVA, M. 1968. Development of vessels in the marginal periodontium in rats. J. dent. Res. 47: 507.

KINDLOVA, M. and MATENA, V. 1962. Blood vessels of the rat molar. J. dent. Res. 41: 650-660.

KOHL, J. and ZANDER, H.A. 1962. Fibres conjonctives oxytalan dans le tissu gingival interdentaire. Paradont. 16: 23-30.

KÖRBER, K.H. 1970. Periodontal pulsation. J. Periodontol. 41: 382-390.

KRIKOS, G.A. 1959. The effects of beta-aminopropionitrile upon the molar teeth of the rat. J. dent. Res. 38: 27-35.

KRIKOS, G.A. 1965. Histochemical studies of the periodontal ligament in lathyritic rats. Archs oral Biol. 9: 415-420.

KRIKOS, G.A., MORRIS, A.L., HAMMOND, W.S. and McCLURE, H.H. Jr. 1958. Oral changes in experimental lathyrism (odoratism). Oral Surg., Oral Med., Oral Path. 11: 309-321.

KRIKOS, G.A., BELTRAN, R. and COHEN, A. 1965. Significance of mechanical stress on the development of periodontal lesions in lathyritic rats. J. dent. Res. 44: 600-607.

LEVENE, C.I. 1967. Experimental osteolathyrism. In: *The Connective Tissue*, (Edited by Wagner, B.M. and Smith, D.E.), International Academy of Pathology Monograph No. 7, pp. 132-141, Williams and Wilkins, Baltimore.

LEVENE, C.I. and GROSS, J. 1959. Alterations in state of molecular aggregation of collagen induced in chick embryos by B-aminopropionitrile (lathyrus factor). J. exp. Med. 110: 771-790.

LEWIN, A. 1970. The direction of tooth movement within the periodontal space. J. dent. Assn. Sth. Africa, 25: 333-341.

LEWIS, H.B. and SCHULERT, A.R. 1949. Experimental lathyrism in the white rat and mouse. Proc. R. Soc. exp. Biol. Med. 71: 440-441.

LILLIE, R.D. 1965. Histopathologic Technic and Practical Histochemistry. 3rd ed., McGraw-Hill, New York.

LILLIE, R.D. 1969. Exploration of dye chemistry in Taenzer Unna orcein type elastin staining. Histochemie. 19: 1-12.

LÖE, H. and NUKI, K. 1964. Observations on the peracetic acid-aldehyde fuchsin (oxytalan) positive tissue elements in the periodontium. Acta odont. Scand. 22: 579-588.

LOEWENSTEIN, W.R. and RATHKAMP, R. 1958. The sites for mechano-electric conversion in a Pacinian corpuscle. J. gen. Physiol. 41: 825-845.

LUKE, D.A. 1975. Oxytalan-type fibres in the developing human and equine temporomandibular joint. J. dent. Res. 54: 1088.

LUNA, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed., McGraw-Hill, New York.

MANDER, S.T.K., MANDER, L.N., CARMICHAEL, G.G. 1968. The staining mechanism of aldehyde-fuchsin, with reference to the oxytalan fibre in the mouse. J. Histochem. Cytochem. 16: 480-485.

MARKENS, I.S. and OUDHOF, H.A.J. 1976. The occurrence and function of oxytalan fibres. Ned. T. Tandheelk. 83: 6-17.

McCALLUM, H.M. 1958. Lathyrism in mice. Nature (London) 182: 1169-1170.

McCALLUM, H.M. 1965. Experimental lathyrism in mice. J. Path. Bact. 89: 625-636.

MELCHER, A.H. and EASTOE, J.E. 1969. The connective tissues of the periodontium. In: *Biology of the Periodontium*, (Editors Melcher, A.H. and Bowen, W.H.), pp. 167-343, Academic Press, London.

MELCHER, A.H. and WALKER, T.W. 1976. The periodontal ligament in attachment and as a shock absorber. In: *The Eruption and Occlusion of Teeth*, (Edited by Poole, D.F.G. and Stack, M.V.), pp. 183-192, Butterworths, London.

MELOAN, S.N. and PUCHTLER, H. 1975. A methanol resorcin-fuchsin stain for elastic tissues and nuclei. Stain Technol. 50: 367-370.

MENZIES, D.W. and MILLS, K.W. 1957. The aortic and skeletal lesions of lathyrism in rats on a diet of sweet pea. J. Path. Bact. 73: 223-237.

MIAKE, K., HIGASHI, S., MACHIDA, N., ITO, S. and OGAWA, T. 1970. Elastic fibres in the periodontal membrane and the cementum in deciduous teeth. Bull. Tokyo Dental Collage, 11: 235-254.

MILLINGTON, P.F., GIBSON, T., EVANS, J.H. and BARBENEL, J.C. 1971. Structural and mechanical aspects of connective tissue. In: *Advances in Biomedical Engineering*, (Edited by Kenedi, R.M.), Vol. I, pp. 189-248, Academic Press, London.

MOMENT, G.B. 1969. Deteriorated paraldehyde: an insidious cause of failure in aldehyde-fuchsin staining. Stain Technol. 44: 52-53.

MOSS, J.P. and PICTON, D.C.A. 1974. The effect of approximal drift of cheek teeth of dividing mandibular molars of adult monkeys *(Macaca irus)*. Archs oral Biol. 19: 1211-1214.

NAOUMENKO, J. and FEIGIN, I. 1974. A simple silver solution for staining reticulin. Stain Technol. 49: 153-155.

NOBLE, H.W. 1969. The evolution of the mammalian periodontium. In: *Biology of the Periodontium*, (Edited by Melcher, A.H. and Bowen, W.H.), pp. 1-26, Academic Press, London.

NOBLE, H.W. 1973. Comparative functional anatomy of the temporomandibular joint. Oral Sciences Rev. 2: 3-28.

OXBERRY, B.A. 1975. An anatomical, histochemical, and autoradiographic study of the ever-growing molar dentition of *Microtus* with comments on the role of structure in growth and eruption. J. Morph. 147: 337-354.

PARFITT, G.J. 1967. The physical analysis of the tooth-supporting structures. In: *The Mechanisms of Tooth Support*, (Edited by Anderson, D.J., Melcher, A.H. and Picton, D.C.A.), pp. 154-156, John Wright, Bristol.

PARKER, G.R. 1972. Transseptal fibres and relapse following bodily retraction of teeth: A histologic study. Am. J. Orthod. 61: 331-344.

PEARSE, A.G.E. 1968. Histochemistry: Theoretical and Applied. 3rd ed., Vol. I, J. and A. Churchill, London.

PICTON, D.C.A. 1965. On the part played by the socket in tooth support. Archs oral Biol. 10, 945-955.

PICTON, D.C.A. 1969. The effect of external forces on the periodontium. In: *Biology of the Periodontium*, (Edited by Melcher, A.H. and Bowen, W.H.), pp. 363-419, Academic Press, London.

PICTON, D.C.A. and SLATER, J.M. 1972. The effect on horizontal tooth mobility of experimental trauma to the periodontal membrane in regions of tension or compression in monkeys. J. periodont. Res. 7: 35-41.

PIEZ, K.A., MILLER, E.J. and MARTIN, G.R. 1964. The chemistry of elastin and its relationship to structure. Adv. biol. Skin. 6: 245-253.

PROVENZA, D.V., FISCHLSCHWEIGER, W. and SISCA, R.F. 1967. Fibres in human dental papillae. A preliminary report on the fine structure. Archs oral Biol. 12: 1533-1539.

PUCHTLER, H. and SWEAT, F. 1964. Histochemical specificity of staining methods for connective tissue fibres. resorcin-fuchsin and van Gieson's picro-fuchsin. Histochemie 4: 24-34.

PUCHTLER, H., SWEAT, F., BATES, R. and BROWN, J.H. 1961. On the mechanism of resorcin-fuchsin staining. J. Histochem. Cytochem. 9: 553-559.

RANNIE, I. 1963. Observations on the oxytalan fibre of the periodontal membrane. Trans. Europ. Orthod. Soc. pp. 127-136.

RIPPIN, J.W. 1976. Collagen turnover in the periodontal ligament under normal and altered functional forces: I. Young rat molars. J. periodont. Res. 11: 101-107.

ROCHE, P.J. 1972. The interpapillary ligament and oxytalan fibres in the gingival col of children. Aust. Orthod. J. 3: 63-65.

ROSS, R. 1973. The elastic fibre. A review. J. Histochem. Cytochem. 21: 199-208.

SAMPSON, W.J. 1975. Oxytalan Fibres in Marsupial Periodontal Ligaments. Master of Dental Surgery Research Project, The University of Adelaide.

SAMPSON, W.J. and SIMS, M.R. 1977. Oxytalan fibre organization in marsupial mandibular periodontal tissues. J. Morph. In Press.

SARNAT, H. and SCIAKY, I. 1965. Experimental lathyrism in rats: Effects of removing incisal stress. Periodontics 3: 128-134.

SCHATZKER, J. and BRANEMARK, P-I. 1969. Intravital observation on the microvascular anatomy and microcirculation of the tendon. Acta orthopaed. Scand. Supp. 126. 3-23.

SCIAKY, I. and UNGAR, H. 1961. Effects of experimental lathyrism on the suspensory apparatus of incisors and molars in rats. Ann. Dent. 20: 90-99.

SELVIG, K.A. 1963. Electron microscopy of Hertwig's epithelial sheath and of early dentin and cementum formation in the mouse incisor. Acta odont. Scand. 21: 175-186.

SELVIG, K.A. 1968. Nonbanded fibrils of collagenous nature in human periodontal connective tissue. J. periodont. Res. 3: 169-179.

SELYE, H. 1957. Lathyrism. Rev. Canad. Biol. 16: 1-82.

SHACKLEFORD, J.M. 1971. Scanning electron microscopy of the dog periodontium. J. periodont. Res. 6: 45-54.

SHEETZ, J.H., FULLMER, H.M. and NARKATES, A.J. 1973. Oxytalan fibres: Identification of the same fibre by light and electron microscopy. J. Oral Path. 2: 254-264.

SIMPSON, H.E. 1967. A three-dimensional approach to the microscopy of the periodontal membrane. Proc. R. Soc. Med. 60: 537-542.

SIMS, M.R. 1973. Oxytalan fibre system of molars in the mouse mandible. J. dent. Res. 52: 797-803.

SIMS, M.R. 1974. Oxytalan fibre response to tooth intrusion and extrusion. IRCS (Research on: Anatomy and human biology; connective tissue, skin and bone; dentistry and oral biology), 2: 1418.

SIMS, M.R. 1975. Oxytalan-vascular relationships observed in histological examination of the periodontal ligaments of man and mouse. Archs oral Biol. 20: 713-716.

SIMS, M.R. 1976. Reconstitution of the human oxytalan system during orthodontic tooth movement. Am. J. Orthod. 70: 38-58.

SIMS, M.R. 1977a. The oxytalan fibre system in the mandibular periodontal ligament of the lathyritic mouse. J. Oral Path. In Press.

SIMS, M.R. 1977b. Oxytalan meshwork associations observed in histological studies of the periodontium of the mouse mandible. Archs oral Biol. In Press.

SIMS, M.R. 1977c. Oxytalan fibre response to tooth intrusion and extrusion in normal and lathyritic mice: A statistical analysis. J. periodont. Res. In Press.

SMITH, A. 1962. The use of frozen sections in oral histology, Part I, J. med. lab. Technol. 19: 26-38.

SNEDCOR, G.W. and COCHRAN, W.G. 1967. Factorial experiments. In: *Statistical Methods*, 6th ed., pp. 339-380, Iowa State University Press, Ames.

SOAMES, J.V. and DAVIES, R.M. 1975. Elastic and oxytalan fibres in normal and inflamed dog gingivae. J. periodont. Res. 10: 309-314.

SOULE, J.D. 1967. Oxytalan fibres in the periodontal ligament of the *Caiman* and the *Alligator* (Crocodilia, Reptilia). J. Morph. 122: 169-173.

SOULE, J.D. 1969. Tooth attachment by means of a periodontium in the trigger-fish *(Balistidae)*. J. Morph. 127: 1-6.

SWEAT, F., PUCHTLER, H. and WOO, P. 1964. A light-fast modification of Lillie's allochrome stain. Archs Path. 78: 73-75.

TANZER, M.L. 1965. Experimental lathyrism. In: *International Review of Connective Tissue Research*, (Edited by Hall, D.A.), Vol. 3, pp. 91-112, Academic Press, New York.

TEDESCHI, L.G. and SOMMERS, S.C. 1961. Oxytalan fibres in sclerosing hemangiomas. Archs Derm. 84: 178-180.

TEDESCHI, L.G. and SOMMERS, S.C. 1962. Oxytalan fibres - Dermal fibromas and giant-cell tendon sheath tumours. Archs Derm. 85: 527-529.

TKACZUK, H. 1968. Tensile properties of human lumbar longitudinal ligaments. Acta orthop. Scand. Supp. 115: 1-69.

TSURUTA, M., ETO, K. and CHIBA, M. 1974. Effect of daily or 4-hourly administrations of lathyrogens on the eruption rates of impeded and unimpeded mandibular incisors of rats. Archs oral Biol. 19: 1221-1226.

VIIDIK, A. 1973. Functional properties of collagenous tissues. In: *International Review of Connective Tissue Research*, (Edited by Hall, D.A. and Jackson, D.S.), Vol. 6, pp. 127-215, Academic Press, New York.

WEIS-FOGH, T. 1960. A rubber-like protein in insect cuticle. J. exp. Biol. 37, 889-907.

YU, S.Y. and BLUMENTHAL, H.T. 1967. The calcification of elastic tissue. In: *The Connective Tissue*, (Edited by Wagner, B.M. and Smith, D.E.), pp. 17-49, Williams and Wilkins, Baltimore.

ZWEIFACH, B.W. 1972. Biomechanics and physiology. In: *Biomechanics: Its Foundations and Objectives*, (Edited by Fung, Y.C., Perrone, N. and Anliker, M.), pp. 3-13, Prentice-Hall, New Jersey.