A HISTOLOGIC AND RADIOAUTOGRAPHIC STUDY OF MIGRATION PATTERNS IN THE MANDIBULAR MOLARS OF NORMAL AND LATHYRITIC MICE

CRAIG WILLIAM DREYER B.D.S. (ADEL.)

A report submitted in partial fulfilment
of the requirements for the degree of
Master of Dental Surgery

The Department of Dental Health
The University of Adelaide

1980

Awarded in August 1980

TABLE OF CONTENTS

TABLE OF ILLUSTRATIONS

SUMMARY.

Previous studies by Thomas (1965) and Michaeli, Pitaru, Zajicek and Weinreb (1975) revealed that a lathyritic disruption of the collagenous fibres of the periodontal ligament resulted in a retardation of tooth eruption. However, only incidental findings of the pattern of bone deposition and resorption have been reported in relation to tooth migration.

The present study was undertaken to histologically and radioautographically compare the patterns of molar tooth migration in normal and lathyritic mice.

The histologic examination determined a morphologic variation in the periodontal ligament fibres on either side of the alveolar septa of control mice. The relevance of this finding was compared with previous observations reported in the mouse (Dunstan, 1975) and in the human periodontal ligament (Edwards, 1977). Furthermore, no evidence could be found for the existence of transalveolar fibres as proposed by Cohn (1972a) and Johnson (1980).

Radioautographic labelling confirmed the reliability of using Sharpey fibres as histologic indicators

of bone deposition. Using both of these markers bone deposition in control animals was found on distal and buccal facing surfaces of alveolar bone as well as on the alveolar crest. Other alveolar bony surfaces showed evidence of resorption. As a consequence, the present study revealed that in addition to distal and occlusal drift in control mice there was also pronounced tooth migration in a buccal direction.

The initiation of lathyrism by a dietary method produced a gradual and progressive change in the mouse periodontal structures. Disruption of the periodontal ligament was seen to commence in the apical region and extend coronally. Significantly however, after forty days, the supracrestal area remained seemingly unaffected. This was believed to reflect collagen turnover within the periodontal ligament.

Concomittant with the lathyritic periodontal ligament disturbance an alteration appeared in alveolar bone activity. A direct relationship was evident between the development of the lathyritic lesion, a loss of Sharpey and principal fibre continuity, as well as a loss of radioautographic label. These findings indicated a cessation of the distal and buccal vectors of migration during lathyrism. However, occlusal drift was continuing. It was considered that the associated bony changes were a result of the inability of a weakened lathyritic

periodontal ligament to support the tooth against occlusal load.

The apparent continuation of tooth eruption therefore disagreed with the previous findings of Thomas (1965) and Michaeli et al (1975). Various explanations of this finding are discussed with the severity of the lathyritic induction cited as a likely contributing factor. Nevertheless, the collagen contraction theory of tooth eruption becomes untenable (Thomas, 1965; 1976).

The intact nature of the transseptal fibres and the observed altered pattern of tooth migration contradicts the tenet of Picton and Moss (1973) who assign to this fibre system a principal role in approximal tooth drift. Since a disruption of either the transseptal or principal fibres alters the pattern of movement it was considered that the integrity of the entire periodontal ligament was essential for physiologic tooth migration.

It has been noted that a force applied to lathyritic collagen, whether orthodontic or masticatory loading, produces contrasting periodontal effects. It was concluded that the behaviour of lathyritic collagen under stress was incompletely understood and an interesting area for further research.

SIGNED STATEMENT.

To the best of knowledge and belief this report contains no material which has been accepted for the award of any other degree or diploma in any university. Furthermore, it contains no material previously published or written by any other person, except where due reference is made in the text.

C.W. DREYER B.D.S. (Adel.)

ACKNOWLEDGEMENTS.

I gratefully acknowledge the guidance and supervision of Dr. W.J. Sampson, Lecturer in Orthodontics, and Dr. M.R. Sims, Reader in Orthodontics, throughout each phase of this project and in the preparation of this report.

To Dr. A.N. Goss, Senior Lecturer in Oral Pathology and Oral Surgery, who freely permitted the use of the facilities of that department, Mr. D.E. Smale, Senior Laboratory Technician, and Mr. B. Hill and the Histopathology Staff of the Institute of Medical and Veterinary Science, I extend my thanks.

The untiring efforts of Mrs. L. McMahon in the instruction of histologic and photographic procedures were fully appreciated. The use of the photographic facilities of the Dental Department of the Royal Adelaide Hospital was kindly extended by Mrs. L. Zaleski.

I am grateful for the expert work of Mrs. D. Lamont in the typing of this text and also for the support and inspiration from my fellow students.

CHAPTER 1

INTRODUCTION.

Physiologic tooth migration is a variable biologic phenomenon occurring in most dentate animals. Migration has been cited as a major factor in the development of the dentition (Baume, 1950) and of the alveolar processes (Orban, 1976). Although descriptions of bone deposition and resorption within the alveolus provided directional information, the overall concept of tooth migration is unlikely to be limited to bone activity alone. The force of occlusion and forces intrinsic to the tooth have been considered in possible causative roles (Moss, 1976), yet the exact aetiology of migration remains undetermined.

One of the functions of the periodontal ligament is to allow tooth movement through bone while maintaining tooth support and attachment to the alveolus. It is believed that a high turnover (Rippin, 1976) or an intersplicing (Sicher, 1923) of the ligament fibres mediates this function. However, the transseptal group of periodontal fibres has been strongly implicated in tooth migration (Picton and Moss, 1973; Moss and Picton, 1974). The surgical interruption of the transseptal fibres resulted in a disturbance of the rate of approximal tooth drift but not its entire elimination.

The fibres of the periodontal ligament have also been implicated in tooth eruption or migration in an occlusal direction. Studies by Berkovitz and Thomas (1969) and Berkovitz (1971) in which tooth eruption continued after the proximal portion of the rodent incisor was transected and removed suggested the likely involvement of the ligament. In particular, contraction of the collagenous ligament fibres was claimed as a possible eruptive force (Thomas, 1976).

Although the exact mechanisms remain obscure, the involvement of the periodontal ligament in tooth movement is undeniable. In this connection the above studies suggest that the integrity of the various component fibres is of importance. Significant information on tooth migration may therefore be provided by a pathological disturbance of the periodontal ligament fibres.

CHAPTER 2

AIMS OF THE INVESTIGATION.

The physical and chemical properties of collagen may be altered by the use of an osteolathyrogen (Levene, 1973). Since collagenous fibres comprise the major component of the periodontal ligament, it was considered that a lathyritic alteration of these fibres might affect tooth migration.

The present study, therefore, planned to investigate the patterns of molar tooth migration in normal and lathyritic mice.

The direction of migration was to be assessed by remodelling changes in the alveolar bone revealed by:

1. A histologic study of bone deposition and resorption using the patterns of Sharpey fibre associations with these surfaces.

2. A radioautographic study using a topographic labelling method to indicate sites of new bone matrix formation.

As a result of these investigations it was anticipated that additional information might be provided

on the role of the transseptal fibres in tooth migration (Picton and Moss, 1973) and also the role of collagen maturation in tooth eruption (Thomas, 1965).

Furthermore, information regarding the severity and distribution of the lathyritic effect on the periodontal structures might be revealed.

CHAPTER 3

REVIEW OF THE LITERATURE.

THE STRUCTURE OF THE NORMAL PERIODONTIUM.

The teeth are invested by a group of tissues which are collectively called the periodontium. One of the functions of these tissues is to provide support for the tooth (Scott and Symons, 1974; Orban, 1976). Although elastin and oxytalin fibres have been identified, support is believed to be mediated by the attachment of numerous collagenous fibres between tooth cementum and alveolar bone (Kraw and Enlow, 1967).

Attaching collagenous fibres of the periodontal ligament have been divided and classified according to their size and orientation. Goldman (1957), Scott and Symons (1974) and Orban (1976) described a transseptal fibre group as a horizontal tooth-to-tooth collagen fibre system linking the units of the dentition above the level of the alveolar crest. Goldman (1957) suggested that these fibres formed a supporting framework for the interproximal gingival tissue. Picton and Moss (1973) and Moss and Picton (1974) believed that this transseptal group of fibres was responsible for approximal migration of teeth. Experimenting with monkeys, it was found that by exercising this tissue a

reduced amount of tooth movement occurred. Sciaky and Ungar (1961) concluded that these fibres did not escape the effects of lathyrism. The characteristic hyalin-like areas disrupted the fibre pattern. However, Sims (1977) reported that the transseptal fibres were least affected in the lathyritic animal since they maintained their horizontal orientation over the alveolar crest.

Black (1886) first used the term principal fibres to describe large collagenous structures crossing the periodontal space. Waugh (1904) stated that, as these fibres passed directly from tooth to bone, they were orientated parallel to one another except where they deviated for vessels and nerves. Descriptions of principal fibre orientation within the periodontal ligament isolated alveolar crest, horizontal, oblique and apical fibre groups (Melcher and Bowen, 1969; Gianelly and Goldman, 1971; Scott and Symons, 1974). Although the exact reason for this orientation was unclear it was suggested by Grant, Stearn and Everett (1968) that these fibres served to anchor and suspend the tooth in its bony socket.

It was believed that the principal fibres extended between, and were embedded in, cementum and alveolar bone (Melcher and Bowen, 1969). Zwarych and Quigley (1965) reported that there were more fibre

insertions into cementum than into bone, a feature they attributed to a uniting of fibres as they crossed the periodontal space. Furthermore, it was suggested that principal fibres embedding into cementum and alveolar bone were Sharpey fibres (Zwarych and Quigley, 1965; Melcher and Bowen, 1969; Quigley, 1970; Gianelly and Goldman, 1971).

Sharpey (1956, cited by Quigley, 1970) first described connective tissue fibres inserting into bone. Sicher and Weinmann (1944) considered Sharpey fibres as extensions into bone of connective tissues at the site of muscle and tendon attachments. Ham (1965) stated that Sharpey fibres in the periodontal ligament were a bone-to-tooth fibre system and Zwarych and Quigley (1965) reported the continuity of these fibres from the alveolar bone to the cementum of the tooth. Quigley (1970) preferred the name "perforating fibres" to describe principal fibres piercing bone. His examination of rats revealed a spiral configuration of these fibres across the periodontal space, with branching as they approached the bony surface.

Zwarych and Quigley (1965) described the insertions of Sharpey fibres more deeply into one side of the interdental septum than on the other. Fibres were found deeply embedded into the distal facing surface of rodent alveolar bone but not so deeply embedded in the

mesial facing surface. The morphology of Sharpey fibres was believed to reflect tooth movement in a distal direction (Zwarych and Quigley, 1965; Kraw and Enlow, 1967).

The literature revealed no information on the effects of lathyrism on Sharpey fibres.

Quigley (1970) reported that some Sharpey fibres passed directly through the interdental septum and termed them "transosseous fibres". Transosseous fibres were histologically examined in the alveolar bone of the mouse by Cohn (1972a), who subsequently challenged the conventional concept of the attachment of Sharpey fibres. Cohn (1972a) described perforating fibres that were continuous through the interdental septum with fibres of the periodontal ligament of the adjacent tooth and designated them as transalveolar fibres. A particular orientation of the oblique system of fibres was noted as they arched occlusally into bone and, therein, turned apically to insert into the adjacent tooth. Cohn (1972b, 1973, 1975) also found similar patterns in the interdental septum and interradicular bone of marmosets, monkeys (Macaca mulatta) and humans, while recently Johnson (1980) described transalveolar fibre development in mice.

Dunstan (1975) detected many fibre bundles passing through the interdental septum and interradicular

bone. In distinction to the regular arrangement recorded by Cohn (1972a), he reported a much more variable pattern of these fibres as they penetrated bone. His investigation revealed that the teeth were more positively and directly connected than was previously thought and, as such, should be considered as a functional group rather than as individual units.

Dunstan (1975) also detected a difference in size of the fibre bundles in the mouse periodontal ligament on either side of the interdental septum. Those adjacent to the distal facing bone surface were consistently thicker than those adjacent to the mesial facing surface, a finding which he attributed to a distal force vector.

Burnett (1978) challenged the concept of a transalveolar fibre system and concluded that such a group was a misnomer. He believed that Sharpey fibres, originally incorporated into the alveolar septum at depository bone surfaces, became uncovered at resorptive bone surfaces. It was suggested that these fibres were not continuous with the fibres of the periodontal ligament adjacent to the resorptive bone surface as proposed by Cohn (1972a). Burnett's (1978) study also correlated the insertion of Sharpey fibres with the direction of tooth migration.

In order to account for tooth movement, Sicher (1923) first proposed the existence of an intermediate plexus within the periodontal ligament. He saw what he believed to be an anastomotic meeting of the principal fibres from the alveolar and cemental surfaces. A remodelling of this fibre anastomosis was deemed to be of functional importance during tooth movement and eruption. Scott and Symons (1974) considered that the intermediate plexus was only present during the active eruptive phase of the tooth and was easily distinguished in continually erupting teeth, such as the rodent incisor. However, under the influence of occlusal forces, the intermediate zone disappeared and the principal fibres ran unbroken from alveolar bone to tooth root.

The existence of the intermediate plexus has remained a point of contention. Opponents to the theory suggested that the plexus was an artifact caused by the tortuous nature of the principal fibres and the plane of section cutting (Trott, 1962). Bernick (1960), in a study of developing rat molars, found no evidence of such a zone. This finding was also supported by Trott (1962) and Zwarych and Quigley (1965) who reported that they were able to trace intact principal fibres across the entire width of the periodontal space. They found no evidence for the presence of the intermediate plexus in the periodontal ligament of the mouse.

Kraw and Enlow (1967) demonstrated histochemically that collagen in the proposed intermediate plexus zone was less mature. It was suggested that one function of this area might be to adjust the length of principal fibres by linking mature fibres to newly formed fibrils. Hindle (1967) also indicated the immature nature of plexus collagen with the aid of polarizing light microscopic techniques.

Gianelly and Goldman (1971) believed that it was not necessary for the intermediate zone to be located in the middle of the ligament. However, in order to rationalize tooth movement, the existence of an adjustment zone was considered essential, whether it be a spliced arrangement of collagen or a higher rate of fibre turnover.

The penetration of the periodontal ligament fibres into the alveolar bone of the tooth socket was proposed by Scott and Symons (1974) to mediate tooth support. Fibre embedded bone was termed "bundle bone" by Stein and Weinmann (1925). Noyes (1953) claimed that the distribution of bundle bone depended on the direction of tooth migration. Such bone was found in areas where recent bone formation had occurred as a result of tooth movement.

Storey (1972) considered that bone was a highly adaptable tissue which was constantly involved with the process of remodelling in order to maintain form. It was

suggested that normal functional stresses induced changes in bone which were reflected by appropriate additions and removal of tissue. By a process of surface apposition and resorption, Scott (1968) described how alveolar bone was able to grow vertically, laterally and in accordance with the direction of tooth migration. Ritchey and Orban (1953) proposed that the position and shape of the alveolar crestal bone depended on the degree of eruption, angulation and position of the related teeth.

Scott and Symons (1974) characterized bone deposition by a line of osteoblasts forming an epithelial-like layer on the surface of newly formed bone. Layered bone deposition produced incremental lines during periods of rest or quiescence. Each new layer of bone incorporated periodontal ligament fibres which were renewed by fibroblastic activity. Kraw and Enlow (1967) also reported the entrapment of ligament fibres at depository bone surfaces. Furthermore, it was suggested that bone deposition maintained the width of the periodontal space.

Kraw and Enlow (1967) also described resorptive bone surfaces. The characteristics of these areas were scalloped margins and numerous osteoclasts. Scott and Symons (1974) located these cells in resorption bays or Howship's lacunae which produced the irregular bony outline. Kraw and Enlow (1967) also described a discontinuity of periodontal ligament fibres at resorptive bone

surfaces. However, it was reported that in some regions fibres were not detached and provided a continuing link between bone matrix and the periodontal ligament at the resorbing bone surface.

LATHYRISM.

The disease of lathyrism has been encountered in medical literature for centuries. Hippocrates (cited by Selye, 1957) described a neurological disorder causing weakness of the extremities and pain in the knee joints. The symptoms appeared secondary to the ingestion of certain varieties of vetch seeds. Comprehensive reviews of the subject have been written (Selye, 1957; Tanzer, 1965; Gardner, 1959a; Levene, 1973; Barrow, Simpson and Miller, 1974). All the above describe lesions involving either the nervous system or the collagenous connective tissues including bone, skin and often blood vessels.

Selye (1957) used the terms neurolathyrism and osteolathyrism to describe two distinct forms of the disease. The first, neurolathyrism, occurred in humans and domestic animals and reached epidemic proportions in Europe and Northern Africa during the eighteenth and nineteenth centuries. Caused by the consumption of various types of lathyrus pea (Lathyrus sativus, Lathyrus cicera, Lathyrus clymenum, Lathyrus latifolius) in times of famine, the disease presented as a neurological

disorder. Levene (1967) noted the manifestation of the disease as a progressive irreversible spastic paraplegia invariably affecting the lower limbs without any sensory disturbance or muscle wasting.

The second form of the disease, osteolathyrism, was deemed by Selye (1957) to be unrelated to the first. The term experimental lathyrism was used interchangeably with osteolathyrism (Levene, 1967) since laboratory animals were affected by both types but apparently not humans. Its isolation as a separate entity has only occurred in recent times. Geiger, Steenbock and Parsons (1933) first reported osseous changes in rats after feeding sweet pea (Lathyrus odoratus) on a percentage weight basis of their diet. Dasler (1954) isolated "toxic crystals" from sweet pea and found that these chemicals produced the same lesions as dietary lathyritic agents. Dupuy and Lee (1954) identified the naturally occurring lathyrogen as β-aminopropionitrile. Ponseti, Wawzonek, Shepard, Evans and Stearns (1956) discovered that other related nitriles (aminoacetonitrile and methylene acetonitrile), whether fed orally or given by injection, produced osteolathyritic changes in young rats. These synthetic compounds caused more severe manifestations of the disease in a shorter period of time. However, Selye (1957) reported that the character of these changes also depended on the age of the animals and the severity of intoxication.

Rather than cause neural disorders, osteolathyrism affected skeletal structures; specifically, tissues of mesodermal origin. Geiger et al (1933) in the first study of lathyrism in rats detailed spinal and sternal curvatures, enlargement of the costochondral junctions, deformities and an abnormal colour of long bones. Selye (1957) also found that there were joint dislocations, prominent muscle attachments, hindlimb paralysis and oral manifestations.

Vascular disorders were also described by Selye (1957), and a separate term, angiolathyrism(Barrow et al 1974), was used to describe these lesions. Since β-aminopropionitrile was found to be the causative agent, exact differentiation from osteolathyrism could not be made. Connective tissue derangement resulted in a weakened vascular wall and aneurysm formation (Selye, 1957). Aortic rupture and subsequent haemorrhage was cited by Barrow et al (1974) as a likely cause of mortality in diseased animals.

THE PATHOGENESIS OF LATHYRISM.

Stamler (1955) determined that the primary effect of lathyrogens was on tissues of mesodermal origin. However, for some time the precise mechanism remained unsolved. Selye (1957), Menzies and Mills (1957), and Gardner, Dasler and Weinmann (1958) suggested that the defect was to be found in tissue ground substance, while

Kennedy and Kennedy (1963) proposed that a blockage of the links between non-collagenous proteins and chondroitin sulphate A and C occurred. Although Menzies and Mills (1957) described an excessive accumulation of ground substance they placed little import on an observed disintegration of collagen fibres.

Histochemical studies by Gardner (1959b; 1960) confirmed a possible relationship between the lathyrus factor and collagen. This was later supported by biochemical studies (Levene and Gross, 1959; Gross, 1963a; 1963b). A quantitative examination using chick embryos by Levene and Gross (1959) revealed that collagen synthesis was not at fault. To account for an increased solubility of collagen they proposed that old, insoluble collagen was transformed into a soluble form under the influence of the lathyritic agent. Gerber, Gerber and Altman (1962) studied the turnover of collagen in lathyritic rats and postulated that there was a failure in the conversion of soluble collagen to the insoluble mature form.

Although Hurley and Ham (1959) found a delay in collagen formation, Fry, Harkness, Harkness and Nightingale (1962) found no difference in the collagen composition of tissues in lathyritic animals assayed for hydroxyproline content. They did find that the tensile strength of collagen was about half that of normal animals.

Tanzer and Gross (1964), employing a radioisotope technique found no evidence supporting a lathyritic interference of collagen synthesis and so confirmed the findings of Gerber et al (1962) and Fry et al (1962).

An explanation for the decreased strength and increased solubility of lathyritic collagen was presented by Martin, Gross, Piez and Lewis (1961) who hypothesized that, under the influence of β-aminopropionitrile, crosslinking in the collagen molecule might be impaired. Although Martin et al (1961) referred to intramolecular bonds, Gross (1963b) suggested that intermolecular links might also be involved.

An initial step in the formation of the collagenous molecular crosslinks is the oxidative deamination of lysine and hydroxylysine in the peptide chains to the semi-aldehydes allysine and hydroxyallysine (Piez, 1968). Crosslinks formed by spontaneous condensation between reactive aldehydes, or between the aldehyde groups and the amide moiety of unaltered lysine or hydroxylysine residues (Tanzer, 1973). Levene (1967) believed that lathyrogens bound to the aldehyde groups on the collagen molecule and blocked the formation of the crosslink. Pinnell and Martin (1968) noted that an enzyme lysyl oxidase was irreversibly inhibited under the influence of β-aminopropionitrile. Bornstein (1970) also concluded that lysyl oxidase function was impaired in lathyrism and

attempted to relate the interference of the cross-linking mechanism to the process of aging. Evidence therefore suggested that enzyme inhibition in the process of collagen crosslinking and maturation was the mechanism of lathyrism.

While the involvement of lysyl oxidase was recognised its actual inhibitory role was not known. Page and Benditt (1972) postulated several possible mechanisms yet determined that β-aminopropionitrile binding to collagen was unlikely. In a biochemical study they ascertained that cyanoacetaldehyde, a metabolic intermediate of β-aminopropionitrile, bound to collagen and β-aminopropionitrile bound to lysyl oxidase and provided a possible molecular basis for the crosslinking defect.

Due to its principal effect on humans, studies on neurolathyrism were infrequent in the recent literature. Ressler (1962) tested β-cyano-L-alanine for possible neurologic activity and found that this amino acid nitrile was neurotoxic for rats. This compound was believed to be an intermediate in the conversion of asparagine to L-α,γ-diaminobutyric acid. Ressler, Redstone and Erenberg (1961) believed there was a chemical and structural similarity between β-cyano-L-alanine, L-α,γ-diaminobutyric acid and β-aminopropionitrile, the known osteolathyrogen. Bell and O'Donovan (1966) isolated oxalyl derivatives of L-α,γ-diaminobutyric acid from Lathyrus latifolius and

also identified β-N-oxalyl-γ, β-diaminopropionic acid as being neurotoxic. Barrow et al (1974) stated that the mechanism of action of the neurolathyrogens was still undetermined. However, it was known that β-aminopropionitrile had no neurologic effect in experimental animals.

THE ORAL MANIFESTATIONS OF LATHYRISM.

The mesodermal changes that occur in lathyrism are manifest at the dento-facial level. Selye (1957) reported exostoses and prominent muscle attachments on the mandibles of rats as well as in other rat skeletal sites. Detailed descriptions of the tissue changes produced in the dental structures have been given by Gardner et al (1958), Krikos, Morris, Hammond and McClure (1958) and Sciaky and Ungar (1961).

Accompanying apical deformation of the molar roots, Gardner et al (1958) reported striking changes within the periodontal ligament. The ligament was narrower than in normal animals and also underwent what was described as a partial hyalinization. Accumulations of homogeneous eosinophilic material were separated by rows of altered fibroblasts. According to Krikos et al (1958) the entire periodontal ligament was affected except for the root bifurcation area.

The origin of the hyalin-like material was disputed. Although ground substance was known to increase

in quantity (Tanzer, 1965; Gardner et al 1958), Krikos et al (1958) and Tanzer (1965) believed that the hyalinized area comprised altered collagen since the staining reactions were similar. Gardner (1960) investigated the histopathologic and cytopathologic pattern of lathyrism by evaluating various histochemical stains and their effect on the periodontal ligament. Although there was an anatomic likeness of these lathyritic deposits to fibrinoid and amyloid, Gardner (1960) concluded that the material was non-fibrous collagen.

A variation in fibroblastic activity was described by Gardner et al (1958), Krikos et al (1958) and Gardner (1960). Within the periodontal ligament fibroblasts were found to increase in size and exhibit an increased basophilia. With their long axes aligned occluso-apically these cells aggregated to form rows or pallisades across the width of the periodontal space. Rows of fibroblasts separating the hyalin-line material produced the characteristic lesions of lathyrism which Krikos et al (1958) and Sims (1977) believed to be an early sign of the disease.

Further histochemical studies of the periodontal ligament were performed by Krikos (1964). His observations of enzyme treated and variously stained sections of lathyritic rat periodontal ligament revealed a fine disorientated network of collagen fibres which did not form fibre bundles. Furthermore, there was an increased area

of tissue ground substance which appeared associated with the changes in the collagen fibres.

Sciaky and Ungar (1961) described the fibre disorientation to be progressive in nature. A decrease in the number of fibre bundles with distinct separation of the fibres themselves was a feature. Most disorganization appeared in the region of the intermediate plexus which was significantly widened. The tissue changes described by Sciaky and Ungar (1961) were similar to those previously reported except for an increase in severity.

An important effect of lathyrism documented by Thomas (1965) was a retardation in the impeded and unimpeded eruption of rodent incisors and molars. From this observation it was believed that collagen synthesis and subsequent maturation provided a tractional eruptive force. Further collaborative evidence was produced by Berkovitz (1974) who found that a deficiency in vitamin C, an essential prerequisite for collagen synthesis, also decreased the eruption rate of guinea pig incisors. Sarnat and Sciaky (1965) and Berkovitz, Migdalski and Solomon (1972) were unable to reach this conclusion since they disclosed no significant disturbance of eruption of unimpeded rat incisors although the impeded eruption rate was affected.

However, Michaeli, Pitaru, Zajicek, Weinreb (1975) re-examined the eruption rates of lathyritic rat

incisors and re-affirmed the findings of Thomas (1965). They considered that eruption patterns were similar to normal rats and tooth eruption was possible in a lathyritically impaired periodontal ligament.

Although a retardation of tooth eruption had been widely reported, only incidental findings of the patterns of lathyritic bone deposition and resorption had been mentioned in relation to tooth migration. Bone deposition was known to occur in lathyrism as evidenced by exostosis formation in areas of stress (Hamre and Yeagre, 1957). However, in general, bone changes in lathyrism had been described as osteoporotic (Krikos et al 1958; Gardner et al 1958), or lytic (Sciaky and Ungar, 1961). Gardner (1960), Kennedy and Kennedy (1963) and Sarnat and Sciaky (1965) reported an altered pattern of resorption of the interradicular and interdental septa in both mice and rats, yet failed to note alveolar bone deposition.

Barrington and Meyer (1966) described appositional and resorptive bone changes in the interradicular areas of teeth in lathyritic male rats and compared them with bone changes in similar areas of normal rats. It was found that alveolar bone resorption was a characteristic feature of lathyritic animals. Sims (1977) also reported bone resorption on both sides of the interradicular and interdental septa in mice lathyritic for six weeks. The presence of osteoclasts lining the distal facing

surface of the alveolar septa was noticed. With lathyrism of increasing severity Barrington and Meyer (1966) noted an accumulation of these cells towards the crestal area of bone which they believed signified a cessation of distal tooth migration in the rat.

The reason for the changes in alveolar bone activity were not known. Krikos et al (1958) and Kennedy and Kennedy (1963) believed that mechanical stress played an important part in the manifestation of these morphological changes. However, recently Heller and Nanda (1979) applied an orthodontic force between the maxillary incisor and first molar tooth of lathyritic and normal rats. It was found that lathyritic rats formed more new alveolar bone in response to the force. Furthermore, the lathyritic agent, injected β-aminopropionitrile, still produced disorganization of the collagen of the periodontal ligament. Heller and Nanda suggested that the typical histologic response to an orthodontic force still occurred in the presence of a chemically and physically altered periodontium. These findings were in contrast to those of Krikos, Beltran and Cohan (1965) and Sarnat and Sciaky (1965), who found no microscopic lesions in the periodontal ligament when mechanical stress was absent from the teeth of lathyritic animals. It was concluded that mechanical stress was necessary for the pathogenesis of lathyrism in the periodontium.

RADIOAUTOGRAPHY.

Radioautography, a specialized histochemical technique, may be used for detecting radioisotopes based on their ability to affect a photographic emulsion (Norris and Woodruff, 1955). The affected emulsion functions as a micro-detector by permitting visual localization of radio-elements in tissues. As such, the method is another form of vital tissue marking whose usefulness in dental research was revealed by Krutchkoff (1972) in a review of the principles, methods and variables involved. Information on the utilization of specific elements is possible both quantitatively and qualitatively.

Radioautographic studies of the growth of bone have involved the use of Ca^{45} (Lacroix, 1960) and P^{32} (Leblond, Wilkinson, Belanger and Robichon, 1950). These radioactive agents have assisted localization of calcification sites in growing and remodelling bone. Tritiated proline, a radioactive amino acid which may localize in forming collagen, has been used to study the collagenous components of bone matrix (Tonna, 1974; 1975; 1976) and of the periodontium (Stallard, 1963; Crumley, 1964; Carneiro, 1965; Carneiro and Fava de Moraes, 1965; Carneiro and Leblond, 1966; Formicola and Ferrigno, 1966; Kameyama, 1975; Rippin, 1976; 1978). Proline turnover studies have produced diverse results in relation to specific areas of the periodontal ligament.

Formicola and Ferrigno (1966) performed a radioautographic study of the developing rat periodontium using tritiated proline to tag the matrix of dental structures. Labelling was found in the extracellular matrices of bone, cellular cementum, dentin, enamel and along collagenous periodontal ligament fibres. Under low magnification it was found that the label appeared as a black line. However, under high magnification, this line was revealed as a row of black dots. The silver grains in alveolar bone indicated a pattern of bony activity around the developing tooth. With time Formicola and Ferrigno (1966) discovered a change in grain density from the basal areas of bone to areas of periodontal ligament attachment. It was believed that this alteration in label distribution was indicative of tooth eruption and periodontal fibre orientation. Furthermore, after eruption, bone label was located on the distal facing surface of individual tooth alveoli and was interpreted as evidence of distal rat molar migration.

Labelling mice with tritiated proline or glycine, Carneiro and Fava de Moraes (1965) discovered that label first appeared in cellular elements and then in the extracellular spaces. These radioactive amino acids produced a heavy label in relation to collagen producing cells, providing evidence that the tag was mainly in collagen. It was determined that the periodontal ligament had a higher collagen turnover than other dense connective tissues such as tendons, ligaments and gingivae. However,

great variability existed in the distribution of the label in different regions of the periodontal ligament. Carneiro and Fava de Moraes (1965) showed that the apical and crestal fibres of the ligament incorporated more label than horizontal or oblique fibres. Constant renewal of collagen in the periodontal ligament was believed to explain the sensitive nature of this structure to metabolic disturbances. Carneiro (1965) claimed that lateral pressure on the tooth might account for the observed apical and crestal distribution of silver grains.

A fast turnover of proline and glycine in the periodontal ligament has been radioautographically revealed. Stallard (1963) found that connective tissue cells were labelled one half to an hour after the administration of tritiated proline. Furthermore, after four hours the label appeared in the fibrillar portions of the periodontal ligament and organic matrix of bone. The use of a collagenase and a metachromatic stain revealed that the label over collagen was due to labelled proline and hydroxyproline bound to these fibres. The pattern of labelling suggested differential rates of fibre formation. Stallard (1963) observed that fibres in the central and alveolar side of the ligament were labelled more heavily than on the cemental side. Furthermore, he found that fibres in the crestal area appeared to contain a greater accumulation of silver grains than elsewhere. It was suggested that the overall pattern of labelling indicated that new fibrils were being incorporated into pre-existing

collagen fibres, as well as new forming fibres.

Evidence of tooth migration was also presented by Stallard (1963). Distal tooth drift in rats was characterized by a line of silver grains on the distal facing surface of the alveolar socket. Diaz (1978) noted evidence of distal tooth migration in rats as early as 12 hours after the administration of tritiated proline.

By inserting a rubber wedge between the first and second molars in rats, Crumley (1964) artificially reversed the normal pattern of first molar migration and observed the deposition of silver grains within the periodontal structures. The label associated with bone deposition and periodontal activity of this tooth was opposite to that found in control animals. Stallard's (1963) finding of the high rate of collagen formation adjacent to alveolar bone was confirmed by Crumley (1964), who further proposed that this might be a requirement of tooth migration.

Kameyama (1975), examining collagen activity, counted silver grains per unit area of the periodontal ligament, gingiva and dental pulp after the administration of tritiated proline. Collagen turnover in the periodontal ligament was found to be significantly higher than in other areas. Kameyama (1975) explained the difference by a contrast in metabolic activity between the three areas.

Skougaard and Levy (1971) confined their attention to collagen metabolism in the periodontal ligament of the marmoset. Continuous collagen synthesis was found from an area adjacent to the alveolar crest to the root apex. Furthermore, contrary to the findings of Stallard (1963), no differences in rate were found across the width of the periodontal ligament from cementum to bone.

Stahl and Tonna (1977) determined whether differential rates of tissue matrix formation existed across the periodontal ligament during normal function. After administration of tritiated proline, differences were neither seen in matrix formation down the length of the mouse periodontal ligament nor across the width. However, with increasing age of the animal, a decrease in overall tissue matrix production was observed.

Rippin (1976), in a similar study, also showed a high yet consistent rate of collagen turnover across the width of the rat periodontal ligament from cementum to bone. Variations in the rate were found to be dependent on tooth movement and stress placed on the tooth.

Tonna (1971, 1974, 1975, 1976) in a series of topographical radioautographic studies suggested that localization and delineation of regions of growth, remodelling and development of tissues could be observed. Labelled proline was used in this manner independent of

its role in studying tissue turnover. It is in this topographical capacity that tritiated proline will be used in the radioautographic procedures of the present study.

Topographical labelling has previously been used to observe bone remodelling. Tonna (1974) assessed skeletal growth in 5-week old mice. Multiple doses of tritiated proline administered over two weeks produced a series of silver grains in the mouse femur. These bands were coincident with the cellular uptake and turnover of radiotracer and provided an assessment of cellular rates of bone matrix precursor production and deposition.

These serial bands also gave evidence to the amount of bone matrix formation which occurred between each dose of label. Tonna (1976) estimated the daily rate of 35-day old mouse alveolar bone formation to be between 2.13 and 4.56 micrometres, and suggested these values indicated the amount of physiologic tooth migration.

Tonna (1975) employed the topographical labelling technique to compare matrix distribution and production of dental and osseous tissues of 5-week old mice. He found that dental matrix production was significantly higher than bone matrix production and that the label provided a means of compiling accurate topographical maps of skeletal and dental structures.

Tonna (1976) again used this labelling technique with other analyses of "parodontal" bone in mice and reported a decrease in alveolar bone activity associated with increasing animal age. This was consistent with the subsequent results of aging tissue matrix turnover found by Stahl and Tonna (1977) and Rippin (1978). Recently, Garant and Cho (1979a) radioautographically examined the genesis of Sharpey fibres and new bone formation in the mouse periodontium. They administered high intravenous doses of tritiated proline to mice at two day intervals and found a topographical pattern differing from previous reports. As well as longitudinal bands running down the length of the interdental septa additional bands were observed running perpendicular to the usual pattern. It was considered that fibroblastic activity remodelled the Sharpey fibre insertions into bone. Burnett (1978) also found silver grains associated with Sharpey fibres within newly formed bone. He believed the label was incorporated into periodontal ligamental fibres which were later entrapped in bone as Sharpey fibres.

Tonna (1974) found that topographical radioautography was a sensitive technique and preferable to the use of fluorochrome and tetracycline bone labels. Furthermore, the proline label was not lost from matrix until bone was resorbed and did not accumulate in cell nuclei, thereby limiting the potential for radiation damage.

The work of Tonna concluded that the radioautographic labelling technique was a dynamic approach to the recording of skeletal physiologic events and a valuable adjunct in assessing pathologic disruptions to skeletal growth, remodelling and development.

RADIOAUTOGRAPHY RELATED TO LATHYRISM.

Radioautography has been used in the study of experimental lathyrism. Attempts were made to discover the origin of the lathyritic collagen (Tanzer and Gross, 1974; Tanzer and Hunt, 1964), its turnover (Gerber et al, 1962) and also determine the defect causing the lathyritic lesion (Kennedy and Kennedy, 1962; 1963).

Tanzer and Gross (1964) employed several isotopes in attempt to localize the source of lathyritic collagen. They reported no lathyritic interference with chick bone collagen synthesis indicating that total collagen concentration in tissues was not changed. However, there was an alteration in the ratio of insoluble to soluble collagen. It was determined that a portion of the soluble collagen came from newly synthesized protein. Furthermore, a double labelling technique using radioactive proline and glycine also revealed that old insoluble fibres could be extracted. As a result it was suggested that lathyrism affected all stages of maturation.

Tanzer and Hunt (1964) supported the hypothesis that a portion of the lathyritic collagen was recently synthesized by extracting labelled collagen after radioactive amino acids had been injected into lathyritic chick embryos. In addition, they found a wide distribution of tritiated β-aminopropionitrile throughout bone. The lathyrogen was completely removed by saline extraction which indicated that it was not bound to collagen.

By extracting the salt soluble fractions after administering radioactive proline Gerber et al (1962) found there was a difference in specific activity of insoluble collagen between lathyritic and normal rats. Little difference was seen in the activity of the soluble collagen fraction between the two groups which led them to conclude that lathyritic rats synthesized less insoluble collagen than normal rats by the inhibition of the conversion of the soluble to the insoluble form.

Kennedy and Kennedy (1962) used a sulphur-35 label to examine the distribution of sulphated mucopolysaccharides and their relation to the lathyritic lesions. Their findings revealed no evidence of cellular inhibition of ^{35}S-sulphate or any significant accumulation of sulphated mucopolysaccharides in the lathyritic lesions. However, the pattern of distribution was abnormal.

A concurrent investigation into protein

metabolism was also performed by Kennedy and Kennedy (1962) using radioactive methionine and glycine as protein precursors. The labelled amino acids were seen in areas of lathyritic change. This finding, coupled with the fact that sulphated mucopolysaccharides were not affected, led to the conclusion that the lathyritic defect was to be found in fibrogenesis. Hypothetically, failure of the extracellular binding of chondroitin sulphates to protein caused abnormal new fibre formation.

The abnormal pattern of label distribution was again seen by Kennedy and Kennedy (1963). Sulphur-35 activity was found to produce an irregular image around the molar teeth in rats. Alternating light and dark areas were seen in relation to the intercellular lakes of lathyritic material and the pallisades of cells. Bone lining the molar tooth sockets showed a discontinuous surface line of silver grains which was believed related to patterns of bone deposition and resorption.

Labelled amino acid studies by Kennedy and Kennedy (1963) again showed that lathyrism did not inhibit protein synthesis. The distinct silver grain image in the periodontal ligament was found to be similar to that seen using the sulphur isotope. Areas of osteogenesis showed a heavy uptake of glycine which produced an image partly over bone cells and partly over bone matrix.

The results observed by Kennedy and Kennedy (1963) had to be viewed with some caution since they noticed a loss of labelled material from their sections during processing.

Orloff and Gross (1963) attempted to learn how lathyrogenic compounds altered collagen extractability by using C^{14}-labelled β-aminopropionitrile. It was found unlikely that lathyrogens functioned by direct combination with collagen. They hypothesized that lathyrogens in some way altered the collagen structure either by disturbing a metabolic process or by the involvement of an unidentified product.

Radioautographic studies related to lathyrism became infrequent in the dental literature after the mechanism of the disease became known. Medical radioautography concerned itself with lathyritic effects on embryogenesis (Goren, Singh and Pentel, 1977). A lack of information therefore exists concerning the precise topographical distribution of silver grains in the lathyritic periodontium, particularly in relation to alveolar bone.

CHAPTER 4

MATERIALS AND METHODS.

PREAMBLE.

A study of tooth migration involves an examination of alveolar bone and its related structures. The ability to designate bone activity (that is, sites of bone deposition and resorption) is essential in determining the existence of tooth migration and indeed its direction.

Macroscopically, such determinations were made using the gross distribution of alizarin stain (Brash, 1926) or by direct observations of the surface appearance of alveolar bone (Duterloo and Bierman, 1976).

Microscopically, variations in cellular activity were described in relation to bone surfaces (Orban, 1976). Furthermore, a characteristic relationship was reported between Sharpey fibres and bone surfaces at depository and resorptive sites (Zwarych and Quigley, 1965; Kraw and Enlow, 1967; Quigley, 1970; Baron, 1973).

The metabolism of bone constituents with the incorporation of marking agents into the organic or

inorganic phases of forming bone has been a valuable indicator of bone depository sites. Inorganic labels have been provided by alizarin (Hoyte, 1960; 1968) and tetracycline (Bevelander, 1964; Johnson and Mitchell, 1966). Heavy metals such as lead may also localize in forming apatite but technical difficulties accompany their use (Hancox, 1972). The procion dyes have recently been used to tag the organic phase of bone (Prescott, Mitchell and Fahmy, 1968). Moreover, radioactive amino acids are also incorporated into forming bone matrix and make use of the unique structural characteristics of protein collagen. Glycine comprises 33% of collagen and proline and hydroxyproline 25% (Melcher and Bowen, 1969). Hydroxyproline is not found in any other protein and so provides a direct biochemical marker for collagen (Hausmann and Neumann, 1961). The incorporation of proline into the amino acid chains of the collagen molecule and its subsequent conversion to hydroxyproline provides the basis for the topographical labelling technique. Since bone matrix has a high content of proline (Hancox, 1972), Tonna (1974, 1975, 1976) successfully used topographical radioautography to determine bone depository sites. He also considered that surfaces containing no radioautographic label had, by inference, suggested sites of bone resorption.

Osteolathyrism may be induced in the experimental animal either by a dietary method or by injection of the causative agent (Dasler and Milliser, 1957). The

administration of 0.2% aminoacetonitrile, a synthetic analogue of β-aminopropionitrile, was found to provide an effective level of lathyrism by either method. However, a weight for weight combination of sweet pea seed (Lathyrus odoratus) containing the active agent β-aminopropionitrile, mixed with commercial mouse feed has also proved adequate for the induction of lathyrism without causing high animal mortality (Sims, 1977). Because of its cheapness and simplicity it was the method chosen for the present study.

EXPERIMENTAL PROCEDURE.

Four-week old, male white mice obtained from the Waite Agricultural Research breeding stock were used throughout the investigation. All mice were kept in an animal house which had regulated temperature, humidity and lighting. By four weeks of age the first and second molars and by five weeks the third molar had fully erupted and attained functional occlusion (Cohn, 1957; Atkinson, 1972). McCallum (1965) reported that male mice were more susceptible to lathyrism and that young animals were affected more so than old.

The experiment was divided into two parts. The first part comprised a direct histologic comparison of the periodontal fibre and alveolar bone configuration of mice fed a normal diet with those on a lathyritic diet. In the second part radioautography was used to indicate

the sites of bone deposition by proline turnover in control and lathyritic mice. Since the collagen content of bone is high it was anticipated that radioactive proline might localize at sites of bone deposition.

Part 1 : Histology.

Thirty-two mice were used (Table 1). Sixteen were fed a diet of crushed commercial mouse feed (W. Charlick, M. & V. Maintenance Diet) and water, ad libitum. The remaining sixteen mice were fed a lathyritic diet consisting of a homogenous weight for weight mixture of crushed commercial mouse feed and sweet pea seed (Lathyrus odoratus). Four animals from each group were sacrificed at the end of 10, 20, 30 and 40 days after commencement of the diets.

Part 2 : Radioautography.

Due to economic considerations radioautography was limited to sixteen mice (Table 1). Initially all mice received an intraperitoneal injection of L-{3,4-^{3}H(N)} proline; specific activity 2.5 x 10^{4}Ci/mol. (New England Nuclear, Boston, Massachusetts). The dosage was 5μCi/gram of body weight (Appendix 1). The stock solution was diluted with sterile isotonic saline to give an injected volume of 0.3 to 0.5 millilitres. This was administered using a 1 ml. tuberculin syringe and a 26 gauge needle. Sterile syringes and

needles were used in each case. Control animals received an equivalent dose of isotonic saline, injected intraperitoneally.

Ross and Benditt (1965) stated that moderately high blood levels of proline were rapidly achieved after intraperitoneal administration. Consequently, because of the relative simplicity and efficiency of the technique, this route of administration was chosen for the present study.

After receiving the initial injection of tritiated proline, the sixteen mice were randomly divided into two groups. One group of eight mice was placed on a normal diet of crushed commercial mouse feed, while the second group of eight mice was placed on a lathyritic diet of the same composition and consistency as described in Part 1.

Two animals from each group were sacrificed 10, 20, 30 and 40 days after commencement of their diets. However, twenty-four hours prior to sacrifice, all mice received a second 5 μCi/gram intraperitoneal injection of tritiated proline. This time span allowed collagen precursor to pass from cellular to extracellular sites and become incorporated into the proteinaceous components of the periodontal ligament and alveolar bone matrix (Carneiro and Fava de Moraes, 1965; Tonna, 1975).

TABLE 1. THE NUMBER AND DISTRIBUTION OF MICE USED DURING THE EXPERIMENT:

Sacrifice Period (Days)	TOTAL MICE - 48			
	HISTOLOGY - 32		RADIOAUTOGRAPHY -16	
	Control	Lathyritic	Control	Lathyritic
10	4	4	2	2
20	4	4	2	2
30	4	4	2	2
40	4	4	2	2

TISSUE PROCESSING.

The mice were killed by ether inhalation. Their mandibles were immediately dissected out and fixed in Bouin's solution (Appendix 2) for twenty-four hours at room temperature. Grimstone and Skaer (1972) suggested the avoidance of picric acid since they believed it subsequently reacted with the radioautographic emulsion. However, Beertsen and Tonino (1975) determined that Bouin's solution was better than formalin or Carnoy's fluid for fixing specimens for radioautographic purposes. Following fixation, specimens were placed in 70% ethyl alcohol for twenty-four hours to remove excess picric acid. This step was also recommended by Beertsen and Tonino (1975).

To facilitate decalcification the mandibles were cleaned of muscle attachments and excess tissue by scraping.

The specimens were decalcified at room temperature in 10% E.D.T.A. with 7.5% polyvinylpyrolidone and buffered to a pH of 6.95. The completion of decalcification was assessed radiographically and in most instances was achieved in 8 days. The decalcified specimens were trimmed prior to further processing. The incisor tooth was severed flush with the lingual surface of the mandible. This produced a flat lingual surface which assisted embedding and orientation of the blocks for section cutting. The trimmed material was then passed through graded alcohols and double embedded in celloidin and paraffin according to the technique of Peterfi (Culling, 1974; Appendix 3). The specimens were then blocked in paraffin (paraplast) using the Tissue Tech. II tissue embedding system (Lab-Tech Products, Naperville, Illinois).

SECTION CUTTING.

Specimen blocks were mesiodistally sectioned beginning from the lingual aspect using a rotary microtome (Leitz Wetzlar, Midland, Ontario) set at a thickness of 8 micrometres. Horizontal sections were commenced from the occlusal aspect and buccolingual sections from the mesial aspect. Schematic diagrams of the various

planes of section of the mouse molars are represented in Figures 1, 2 and 3. Serial sections were placed on chromic acid cleaned gelatinized slides. Standard histologic technique was followed to ensure complete flatness of sections on each slide.

Section trimming and embedding in paraffin were deemed to be critical steps. Failure to orientate each mandible correctly caused sections to be cut obliquely through the teeth rather than in mesiodistal, horizontal or buccolingual planes. Section cutting in a true mesiodistal plane for all three molars simultaneously was impossible, however, due to an increasing lingual inclination of the molar teeth towards the distal (Fig. 3). Because of the increasing lingual inclination of the molars and the variability involved in the embedding process, the examination mainly involved a study of the first and second molars, which were consistently reproduced. From the horizontal cross-section and limited mesiodistal and buccolingual appearance periodontal morphology of the third molar was inferred.

STAINING.

The main purpose of staining was to reveal the collagenous component of the periodontal tissues. A second objective was to demonstrate Sharpey fibres from bone matrix. Both of these structures contain a high collagen content. A number of stains were therefore

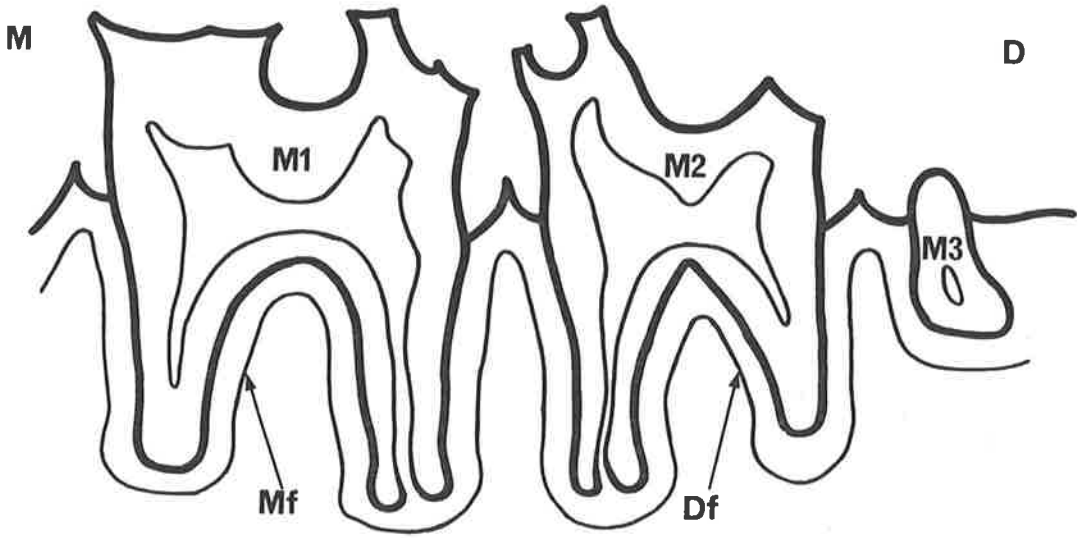


Figure 1. SCHEMATIC DIAGRAM OF THE MOUSE MOLARS M1, M2, M3:

MESIODISTAL SECTION.

M - mesial.

D - distal.

Mf - mesial facing bone surface.

Df - distal facing bone surface.

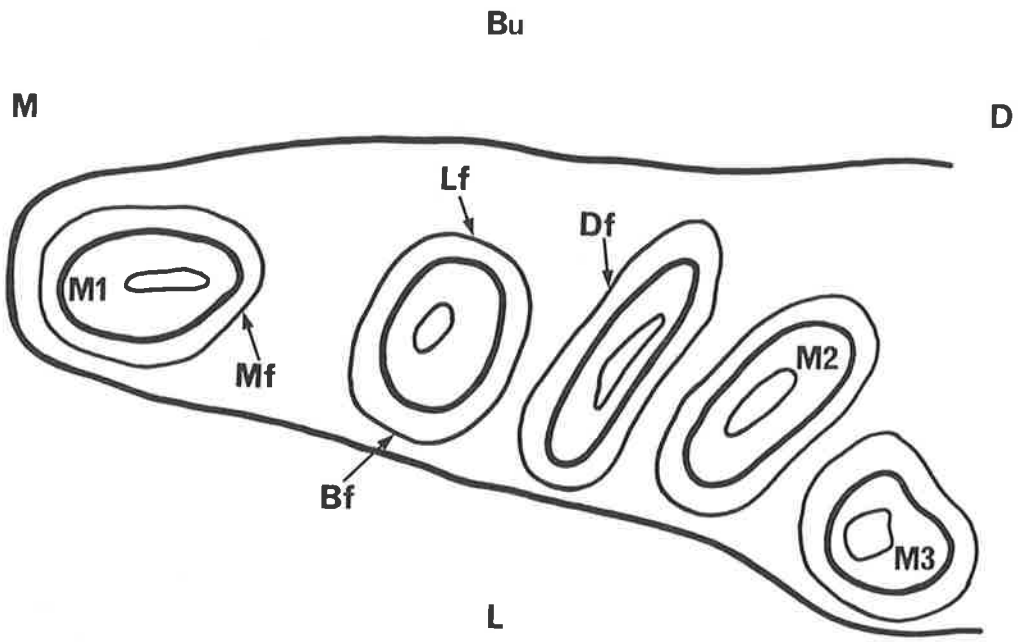


Figure 2. SCHEMATIC DIAGRAM OF THE MOUSE MOLARS M1, M2, M3:

HORIZONTAL SECTION AT THE MID-ROOT LEVEL.

M - mesial.

D - distal.

Bu - buccal.

L - lingual.

Mf - mesial facing bone surface.

Df - distal facing bone surface.

Bf - buccal facing bone surface.

Lf - lingual facing bone surface

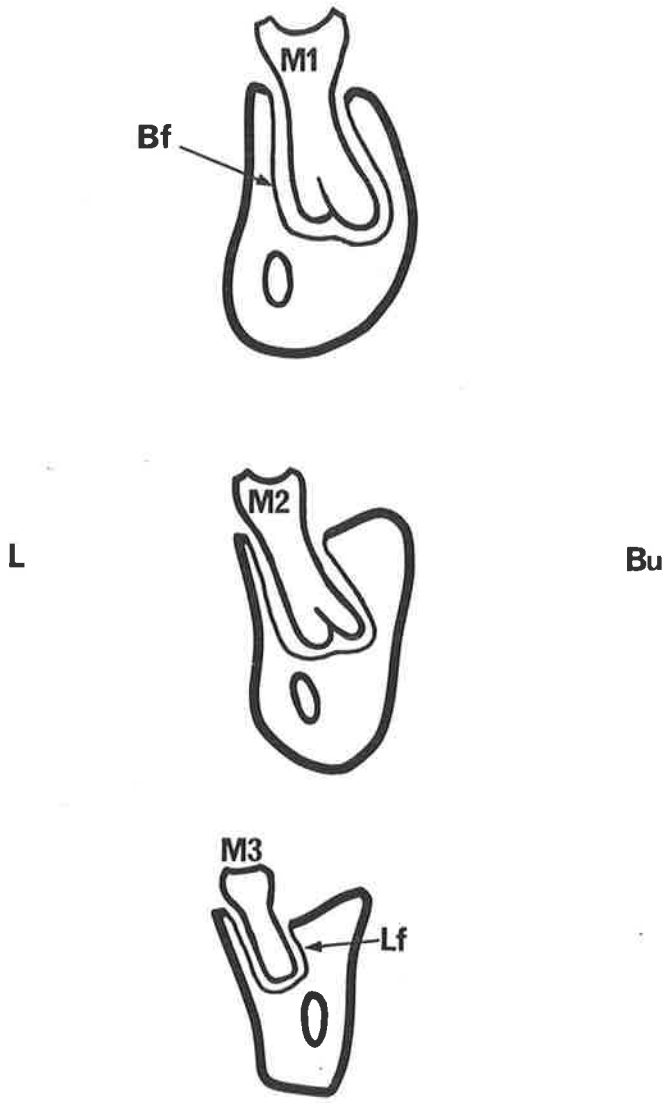


Figure 3. SCHEMATIC DIAGRAM OF THE MOUSE MOLARS M1, M2, M3:

BUCCOLINGUAL SECTION.

Bu - buccal.

L - lingual.

Bf - buccal facing bone surface.

Lf - lingual facing bone surface.

evaluated for their ability to stain collagen. These included Herovici's technique (Herovici, 1963), Pollack's trichrome (Luna, 1968, p.116), Cason's stain for radioautographs (Cason, 1950) and the technique of Moss and Gray for bone incremental lines (Moss and Gray, 1973). Following a trial period, extensive use was made of Herovici's technique for staining collagen. Ehrlich's haematoxylin and eosin (Luna, 1968, p.35) was used to depict cellular elements within the periodontium. After staining, slides were dehydrated in graded ethyl alcohol and mounted in PIX.

RADIOAUTOGRAPHIC TECHNIQUE.

Radioautographs were prepared following the dipping technique of Rogers (1979).

Slides were deparaffinized in two changes of xylene to ensure complete wax removal before being taken to water. Hydrated slides were transported to a photographic darkroom where a temperature of 18^{o}-20^{o}C and a relative humidity of 40%-50% prevailed. In this constant atmosphere the "dipping technique" was performed.

Under safelight conditions 12 millilitres of distilled water and 1 millilitre of glycerol were placed in a coplin jar set in a water bath at a temperature of 43^{o}C. 12 millilitres of molten nuclear emulsion (Ilford K2) were added and the solution gently stirred to ensure

complete mixing. The diluted emulsion was allowed to stand for a short period to permit dispersion of entrapped air bubbles. Periodically a clean slide was dipped into the mixture to test for the presence of air bubbles and the evenness of the coating.

Experimental slides were individually dipped into the emulsion. Excess emulsion was allowed to drain before wiping the back of the slide and placing it on a cooled metal surface. As a test for background radiation, a number of histologic slides not incorporating tritiated proline were similarly "dipped". According to Rogers (1979), this technique and emulsion dilution produces an emulsion layer 3-4 micrometres thick. Coated slides were left in darkness for 45 minutes to dry under a gentle circulation of air.

Dried slides were placed in black light tight boxes with a drying agent (silica gel) and left to expose in a refrigerator at $4^{o}C$.

Exposure was routinely for 35 days at this temperature. Coated slides were then returned to the darkroom and developed in Ilford Phen-X developer for 8 minutes and fixed in 30% sodium thiosulphate. Slides were then ready for staining.

STAINING RADIOAUTOGRAPHS.

Certain problems are inherent in staining radioautography. Rogers (1979) stated that prestaining radioautographs was desirable. However, some stains could be removed by the vigorous nature of the processing. The pH of stains was also critical in poststaining radioautographs since silver grains could be removed by acidic stains or the gelatin coating affected by alkaline dyes (Rogers, 1979).

It was deemed desirable to obtain a direct correlation and comparison of collagen fibre patterns with silver grain distribution. Belanger (1961), Thurston and Joftes (1963) and Burnett (1978) evaluated many stains and recommended those that were compatible with the radioautographs. Stains selected for extensive use were a nuclear fast red, indigo-carmine technique of Mortreuil-Langlois (1962), a connective tissue stain advocated by Cason (1950) and Ehrlich's haematoxylin and eosin (Luna, 1968, p.35).

After staining all slides were dehydrated and mounted in PIX.

PHOTOGRAPHY.

Photomicrographs were taken of selected slides using a Ziess Axiomat (West Germany). Exposure times

were automatically controlled for both black and white and colour films.

For black and white photography, 4" x 5" negatives were produced using Ilford FP4 Plate Film. These were developed using Ilford ID2 developer, fixed in Ilford Hypam fixer and printed on Ilford Ilfospeed paper in accordance with the requirements of definition and contrast.

For colour photography, either 35mm. Kodak photomicrography film 2483 or Ektachrome 200 Daylight film were used. Processing, using the Kodak E4 or E6 process, respectively, produced positive slides from which were printed colour prints using the Ilford Cibachrome technique.

CHAPTER 5

RESULTS.

I. HISTOLOGY.

STAINING.

Most of the histologic stains were specifically chosen for their ability to stain collagenous connective tissue. It was found that some were better than others when used for this purpose. In some instances it was difficult to distinguish the collagen of the principal fibres due to the intense staining of the cellular elements in the periodontal ligament as well. Furthermore, without selective staining it was also difficult to visualize Sharpey fibres as they entered alveolar bone or to differentiate these fibres from bone matrix. The relative merits of the various stains are listed below.

Herovici's Stain (1963):

Herovici's stain proved to be excellent for disclosing the principal collagenous fibres of the periodontal ligament as well as Sharpey fibres penetrating bone (Figs. 11, 18). According to Herovici (1963), his technique stained mature collagen red and immature collagen blue. The differential staining of collagen

fibres was not seen in either control or lathyritic tissue sections. All fibres appeared red and could be followed across the periodontal space. Red Sharpey fibres could be distinguished against the dark pink background of bone matrix. Herovici's technique highlighted the periodontal vascular system which stained bright yellow and also revealed a fine reticulum throughout the ligament. Cell nuclei were not deeply stained and so did not hinder fibre visualization. Excellent contrast was provided for photomicrography, but the technique was not compatible with radioautography.

Pollack's Trichrome (Luna, 1968, p.116):

The staining of collagen was enhanced if nuclear staining using Geimsa's solution was omitted. Differential staining was achieved between principal fibres of the periodontal ligament which stained green and red coloured Sharpey fibres. An alleged stain differential between Sharpey fibres and bone matrix (Burnett, 1978) appeared inconsistent. Staining for one to three minutes was believed to demarcate Sharpey fibres against a green background of bone. Irrespective of the staining time bone matrix was generally coloured red although Sharpey fibre form could be distinguished (Fig. 35). Red fibres in bone changed to green on entering the periodontal ligament. Although staining was irregular, good photographic results were possible but radioautography was unsuccessful.

Ehrlich's Haematoxylin and Eosin (Luna, 1968, p.35):

Haematoxylin and Eosin provided a reference stain for cellular elements. Eosinophilic lathyritic material of the lathyritic lesion was outlined by the rows of basophilic fibroblasts (Fig. 4). The stain was also successfully used on radioautographs (Fig. 57), although silver grains appeared to be fewer in number compared with other staining techniques. The differentiation procedure using acid-alcohol may have accounted for this.

Nuclear fast red, indigo-carmine stain of Mortreuil-Langlois (1962):

This technique was compatible with radioautography. Silver grain topography was highlighted by the pale background colour (Figs. 42, 54). The lack of differential staining made many structures indistinct. Sharpey fibres were poorly defined. However, cellular elements were enhanced by the nuclear fast red. The time of immersion in indigo-carmine was routinely reduced to five seconds in order to prevent overstaining. Photomicrographs of this stain appeared dull, lacked contrast and reproduced poorly.

Cason's Stain (1950):

Cason's stain was a one-step technique which combined Mallory's and Heidenhain's connective tissue stains. Processed radioautographs were successfully

stained using this method, which also permitted the differentiation of Sharpey and principal fibres. The number of silver grains appeared reduced when compared with the Mortreuil-Langlois technique (1962). Cason's technique often deeply stained the gelatin coating on radioautographs. This was removed by prolonged washing. However, the quality of stain was also reduced. Photographic reproduction was marred by the reduced number and the lack of stain contrast with silver grains.

The Stain of Moss and Gray (1973):

Using Biebricht scarlet instead of haematoxylin, this stain was a modification of procedures involving Ehrlich's haematoxylin and eosin technique. Cell nuclei were prominent. However, fibre patterns were not clear. Eosinophilic bundles were apparent in the periodontal ligament although their form could not be followed exactly. Sharpey fibres in the alveolar bone merged with, and became inseparable from, bone matrix. The value of the stain was found in its ability to outline bone. Resorptive and depository surfaces were clearly demarcated as well as incremental lines which traversed the length of the alveolar bone from crest to base. Excellent photomicrographs of bone detail were achieved with this stain.

Several other connective tissue stains were tried including Gomori's aldehyde fuchsin (Luna, 1968, p.78), toluidine blue (Culling, 1974, p.313), Van Geison's stain (Luna, 1968, p.76), Gomori's trichrome (Luna, 1968, p.93), Heidenhain's connective tissue stain (Luna, 1968, p.86), Masson's trichrome (Luna, 1968, p.94) and Saville Bradbury's modification of Lillies' technique (Luna, 1968, p.72) for collagen. Either because they failed to adequately indicate Sharpey fibres, or because they did not improve the results of stains already used, they were not used routinely.

LATHYRISM.

Macroscopic Examination:

All mice survived the experiment, remaining active and seemingly eating well throughout. Gross skeletal deformities were not apparent. Compared with control animals, the excised mandibles appeared normal in shape and size. There was no sign of exostosis formation either on visual or radiographic examination. The only outward sign of irregularity was a loss of coat lustre noticed after three weeks on the lathyritic diet.

Microscopic Examination:

The diet of 50% sweet pea seed mixed with commercial mouse feed proved adequate to initiate the microscopic signs of lathyrism. As distinct from control

animals there was the impression of an increase in tissue vascularity, seen particularly in horizontal sections. The greatest lathyritic disturbance of the periodontium was noticed after forty days. Various dental and periodontal structures were examined in turn.

LATHYRITIC MATERIAL.

From the tenth to the fortieth day changes were seen in the periodontal ligaments of all lathyritic mice as a result of accumulations of amorphous eosinophilic material (Fig. 4). Collections of this material gradually and progressively disrupted the orientation of the principal fibres.

Between these amorphous accumulations the cells of the periodontal ligament gradually aligned producing the pallisading effect described by Gardner et al (1958). Rows of enlarged, more deeply basophilic, fibroblastlike cells were orientated with their long axes directed occlusoapically almost paralleling the normal arrangement of principal fibres (Fig. 4). The alignment of the cell rows, however, was at right angles to the normal arrangement of fibres. An increase in the number of cells appeared associated with the size of the amorphous areas.

The distribution of the lathyritic material became increasingly widespread as the lathyritic period

continued. During the early stages of lathyrism small accumulations of amorphous material were irregularly dispersed throughout the apical third of the periodontal ligaments of the three molars. Mesiodistal and buccolingual sections revealed heavier deposits around the root apices of the teeth and also the beginning of abnormal cell orientation (Fig. 5). Negligible cell orientation appeared in other areas.

As the size of these lathyritic material accumulations increased across the width of the periodontal space the distribution extended from the apical areas towards the alveolar crest (Fig. 6). Cell alignment accompanied the enlargement of the amorphous deposits. Horizontal sections showed that the entire roots were encircled (Fig. 7). At the end of forty days of lathyrism, in neither mesiodistal nor buccolingual sections (Fig. 8) was the lathyritic lesion seen to extend above the alveolar crest and so disturb the supracrestal fibres.

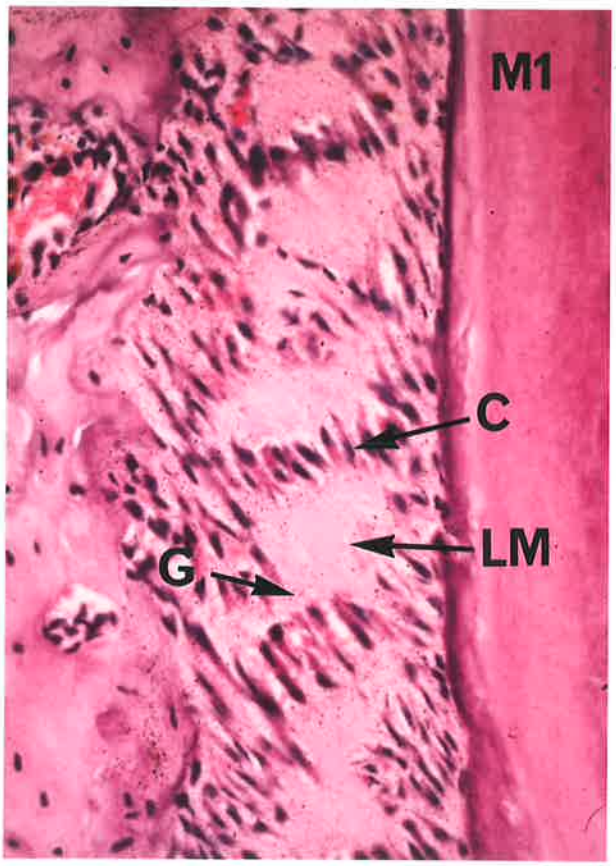


Figure 4. A radioautograph of a 40-day lathyritic lesion in the periodontal ligament adjacent to the first molar (M1) showing the pallisading cells (C) surrounding amorphous material (LM). Silver grains (G) are present within the material adjacent to the cells.

H & E, x450.

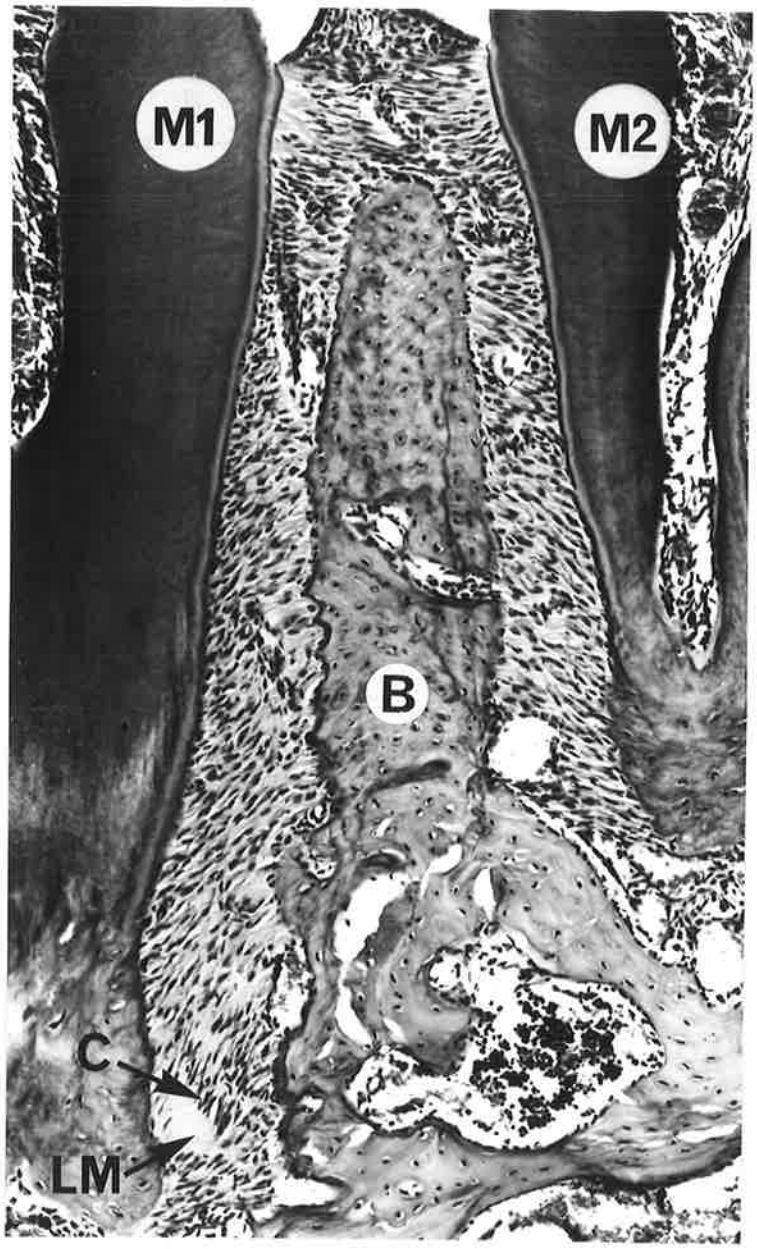


Figure 5. A mesiodistal section of the first and second molars (M1, M2) and interdental bone (B) of a 10-day lathyritic mouse. Lathyritic material (LM) is seen in the centre of the apical periodontal ligament and abnormal cell alignment (C) is also beginning.

H & E, x100.

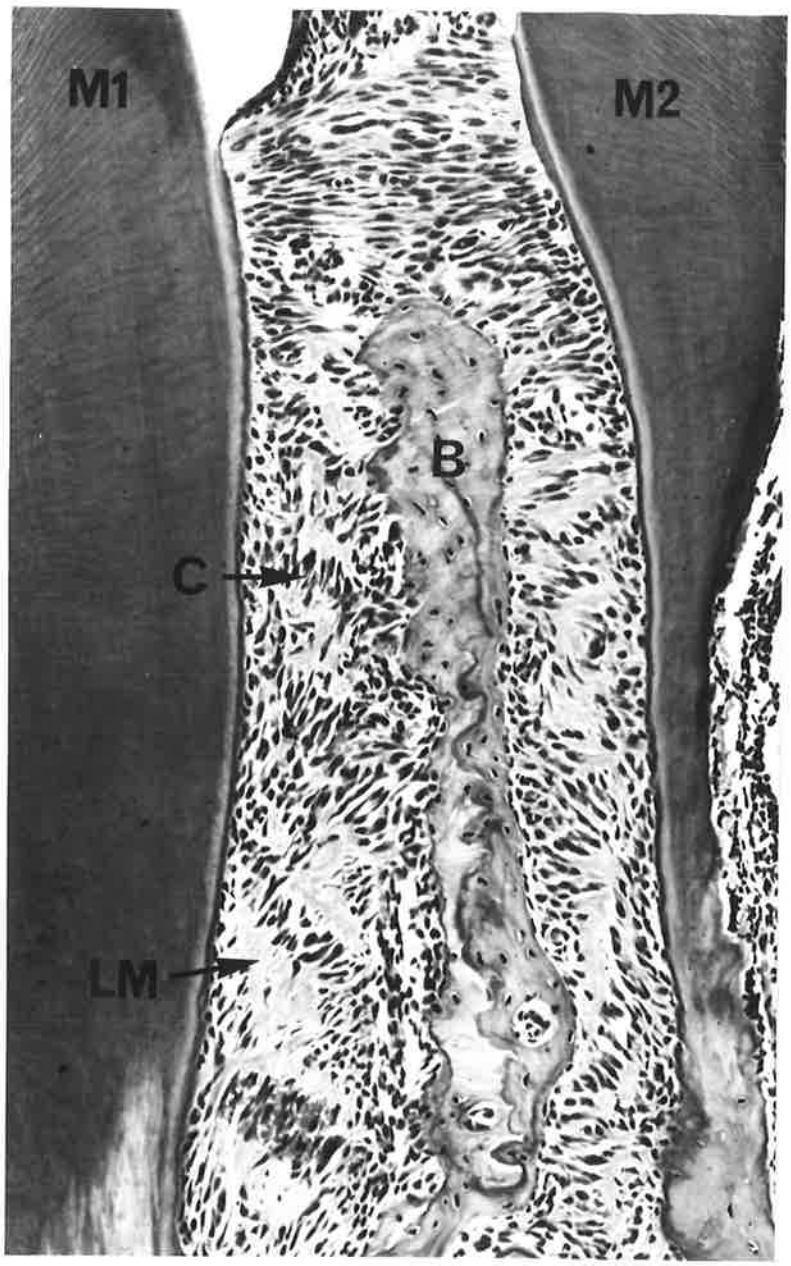


Figure 6. A mesiodistal section of the first and second molars (M1, M2) and interdental bone (B) of a 40-day lathyritic mouse. The lathyritic lesion of amorphous material (LM) and pallisading cells (C) involves most of the subcrestal region but the supra-crestal area is relatively intact.

H & E, x120.

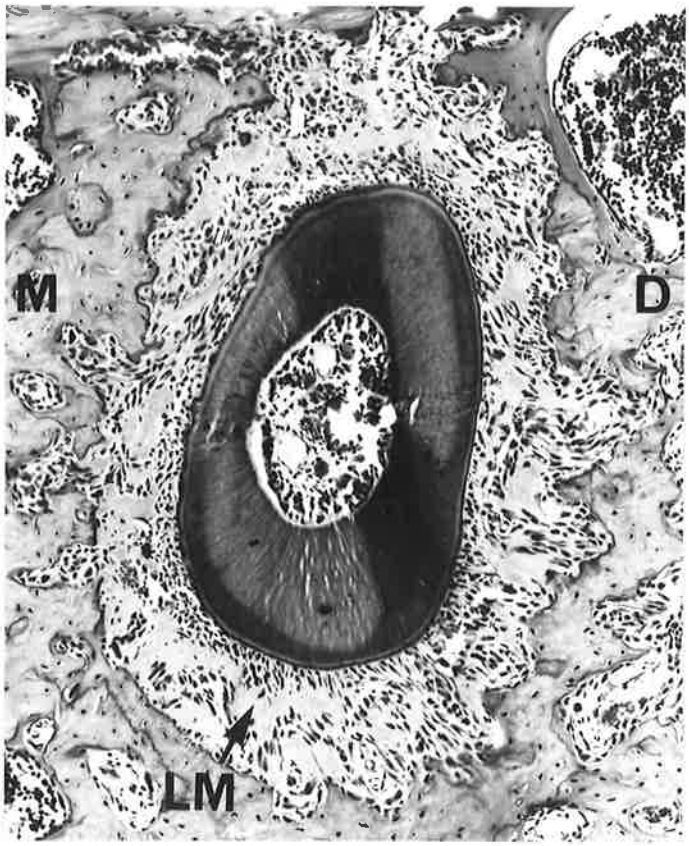


Figure 7. Lathyritic material (LM) encircling the entire distal root of the first molar. M, mesial; D, distal.

H & E, x120.

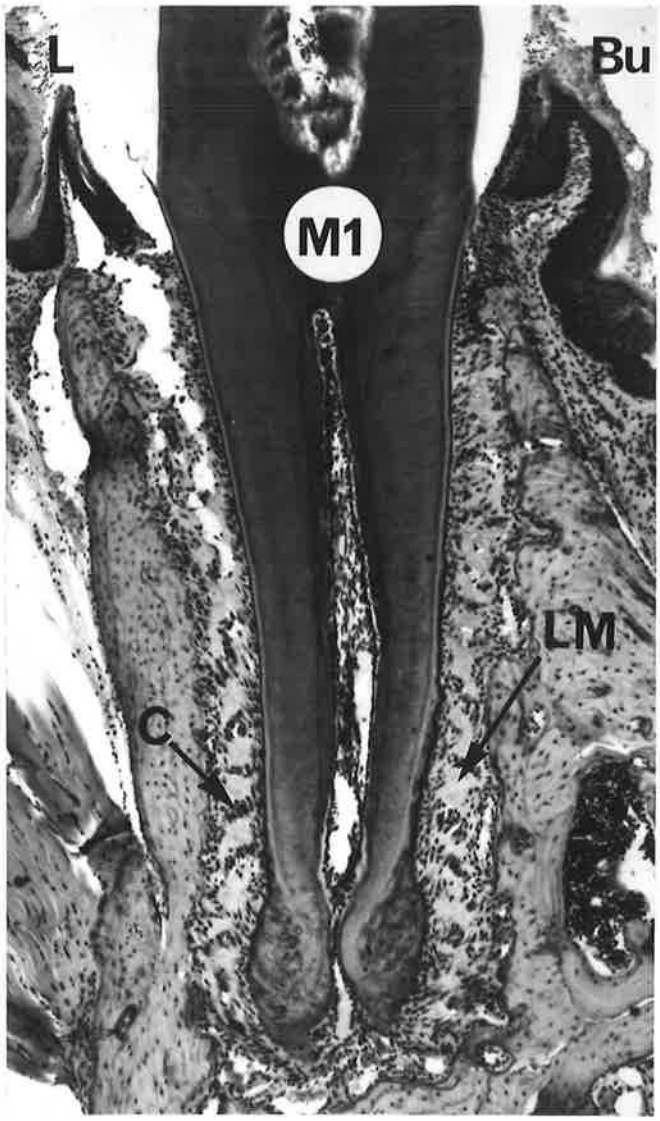


Figure 8. A buccolingual section through the distal root of the first molar (M1) showing the widespread distribution of lathyritic material (LM) and pallisading cells (C) in the 40-day lathyritic mouse periodontal ligament. Bu, buccal; L, lingual.

H & E, x100.

TRANSSEPTAL FIBRES.

Control Animals:

The transseptal fibres comprised a collection of collagen bundles passing over the alveolar crest and beneath the lamina propria of the gingivae. Fibres originated immediately below the cementoenamel junction of one tooth and passed to similarly insert in the neighbouring tooth (Fig. 9). Fibre bundles were well aligned as they joined adjacent teeth. Much branching and intermingling of fibres was evident such that individual fibres could not be traced all the way between adjacent cemental surfaces. Often at the point of fibre insertion there appeared a slight thickening of the cemental layer.

Cells related to the fibres were spindle-shaped and orientated with their long axes parallel to the direction of the fibres. Horizontal sections confirmed the well-aligned and direct nature of this fibre system. The buccal and lingual extremities of the system were seen to correspond with the buccal and lingual borders of the teeth so that fibres were contained interdentally in a mesiodistal direction.

TRANSSEPTAL FIBRES.

Lathyritic Animals:

Lathyrism produced no appreciable changes in

the transseptal fibre system during the forty-day experimental period (Fig. 10). Cemental surfaces of adjacent teeth were still linked by a discrete band of tissue above the alveolar crest. Intermingling and branching of the fibres was still evident as orientation was maintained. Cellular elements also paralleled fibre direction. Thickening of the cemental surface was again noticed at the insertion of the fibre bundles. These cemental spurs appeared to be slightly larger compared with those seen in control animals.

In horizontal sections fibre alignment was confirmed.

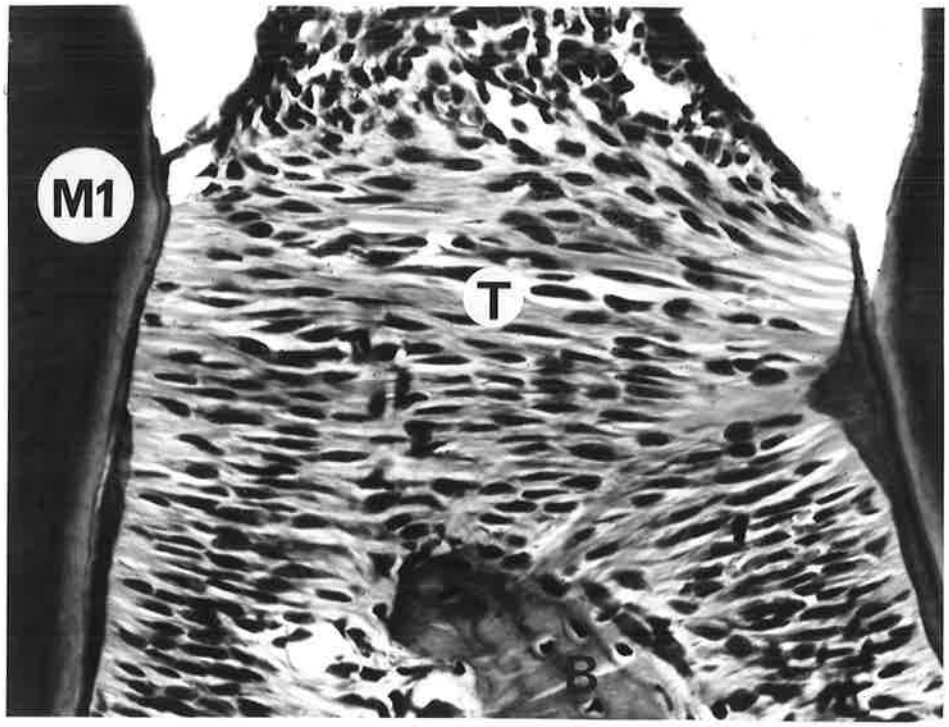


Figure 9. Transseptal fibres (T) of a 40-day control mouse. Both the fibres and cells are well aligned between adjacent cemental surfaces. M1 - first molar, B - alveolar crest.
H & E, x400.

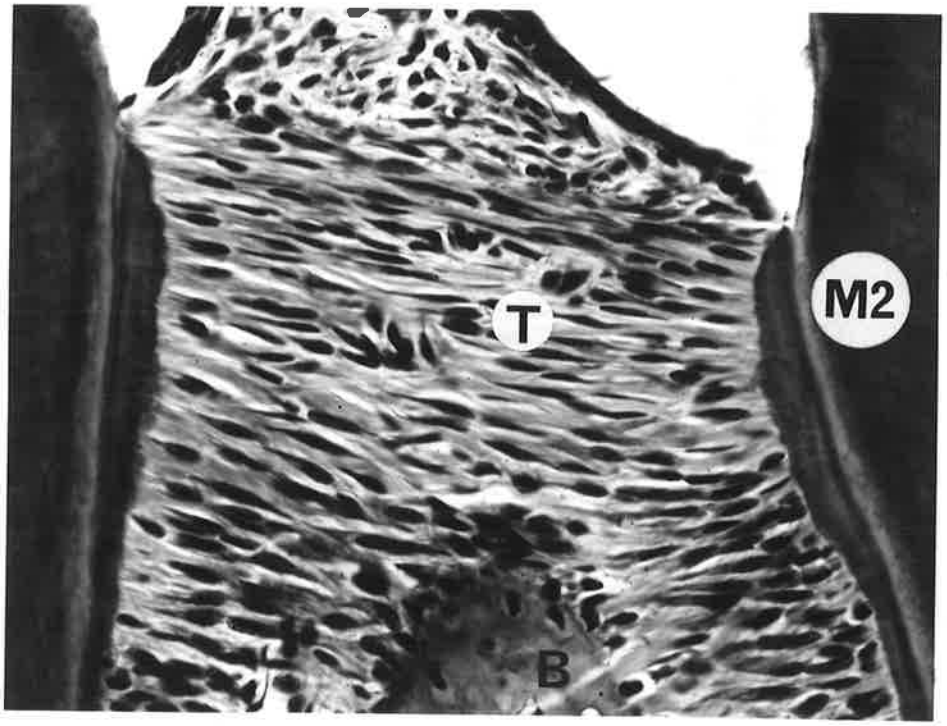


Figure 10. Transseptal fibres (T) of a 40-day lathyritic mouse. Fibres maintain their orientation and there appears to be no cell pallisading. An increase in hypercementosis is apparent. M2 - second molar, B - alveolar crest.
H & E, x400.

PRINCIPAL FIBRES.

Control Animals:

The periodontal ligament comprises masses of fibres extending from cementum to alveolar bone (Fig. 11). The appearance of these fibres and the width of the periodontal space remained remarkably constant throughout the control animals.

The largest of these fibres, termed principal fibres, maintained within the ligament a particular orientation which appeared dependent on their level of origin (Fig. 11). Fibres arising from the cemental surface adjacent to the alveolar crest coursed slightly apically towards alveolar bone. Principal fibres arising at the mid-root level ran horizontally while apical fibres ran obliquely in an occlusal direction towards bone.

The morphology of the principal fibres varied depending on their relation with alveolar bone. The periodontal ligament between mesial facing bone surfaces and the tooth root consisted of fine wavy fibres (Fig. 12). Dividing after their cemental origin, these structures continued to branch and communicate with their neighbours in a delicate network. There appeared no definite union of fibres prior to attachment to bone (Fig. 12).

The periodontal ligament between distal facing bone surfaces and the tooth roots was comprised of larger principal fibres (Fig. 13). Less division after leaving cementum produced a larger fibre which appeared to branch and intercommunicate less freely with its neighbours. Nearing alveolar bone, fibres united to form fibre trunks (Fig. 13). These were large in size (diameter) with the largest found in the alveolar crest region. The size decreased towards the middle and apical areas of the ligament. However, the size of principal fibres adjacent to distal facing bone surfaces remained greater than fibres associated with mesial facing bone surfaces.

In horizontal sections principal fibres emanated from the tooth root like spokes of a wheel (Fig. 14). A direct yet wavy course from cementum to bone was apparent. The size difference between fibres was again seen in relation to the bony surfaces. Also, this plane of section coupled with views in a bucco-lingual plane (Fig. 15) revealed that large fibre trunks were associated with buccal facing bone surfaces, whereas fine fibres were associated with lingual facing surfaces.

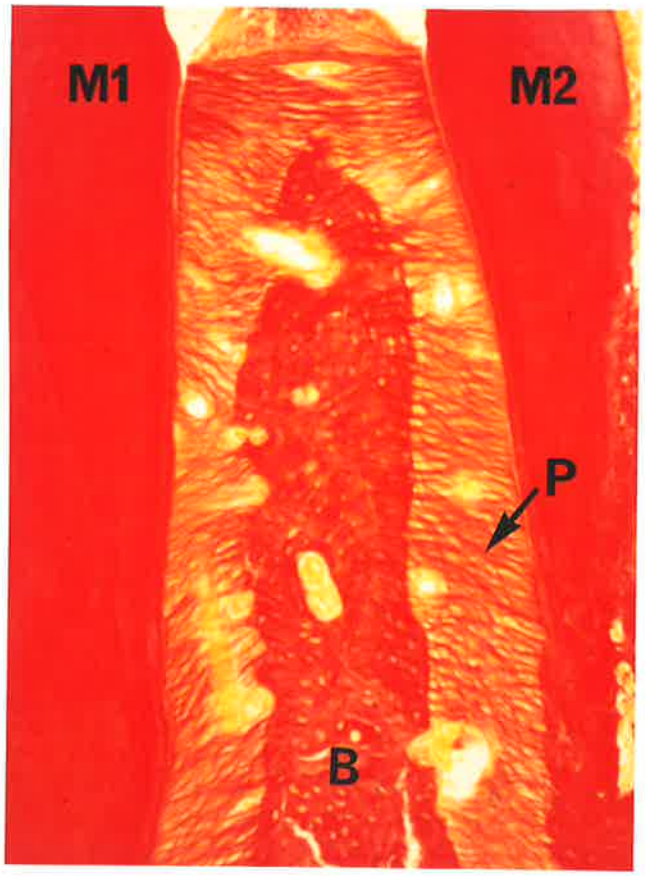


Figure 11. Principal fibres (P) are seen linking the first and second molars (M1, M2) with the interdental septum (B) in a 40-day control mouse. The changing fibre orientation from the crest to the apical region is evident.

Herovici, x120.

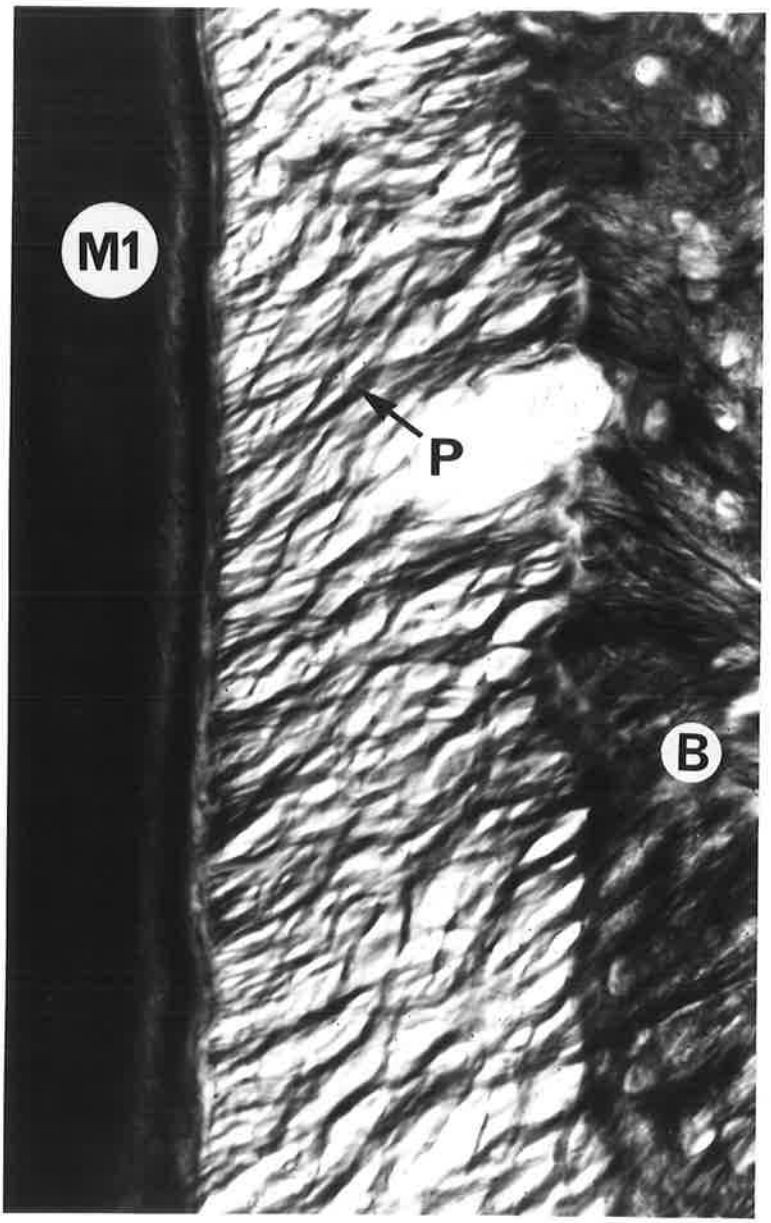


Figure 12. Fine, delicate, intercommunicating principal fibres (P) of the periodontal ligament are shown linking the first molar (M1) to the mesial facing surface of the interdental septum (B) in a 40-day control animal.

Herovici, x450.

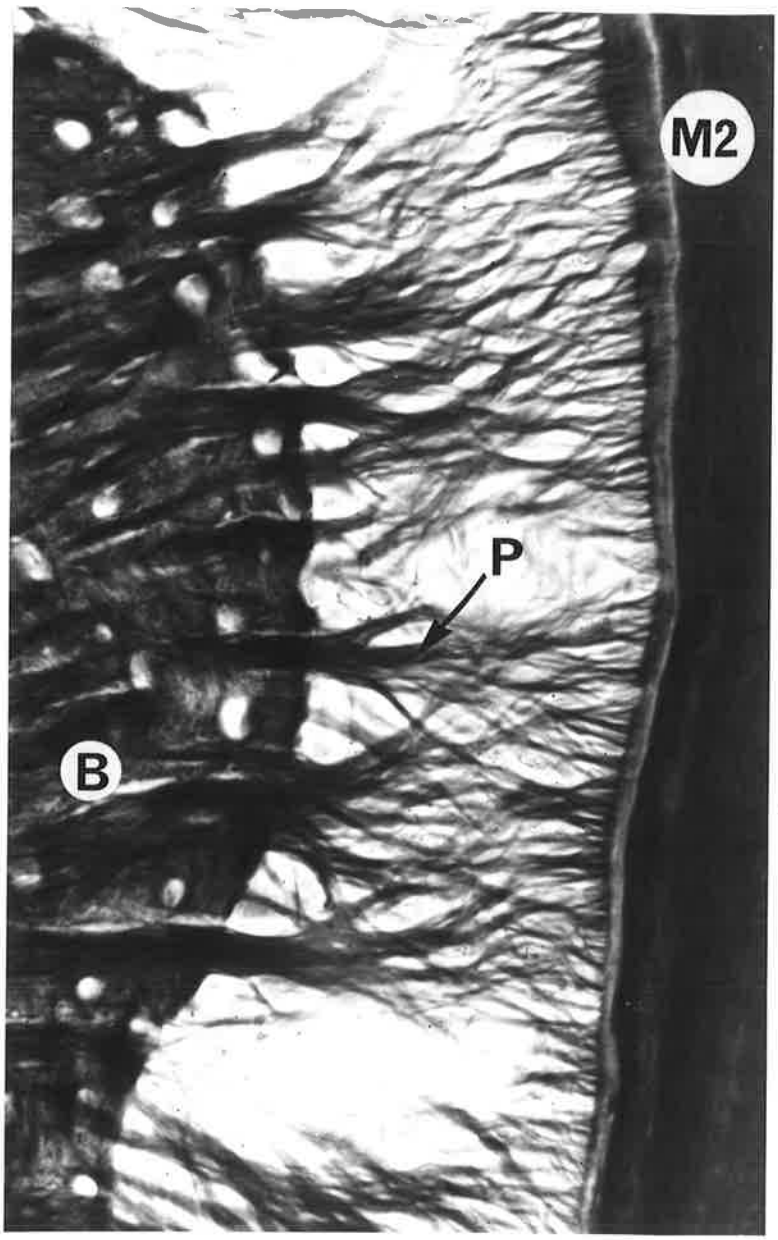


Figure 13. Large principal fibres (P) are seen originating from the second molar (M2) and forming large fibre trunks before entering the distal facing bone surface of the interdental septum (B) in a 40-day control animal.

Herovici, x450.

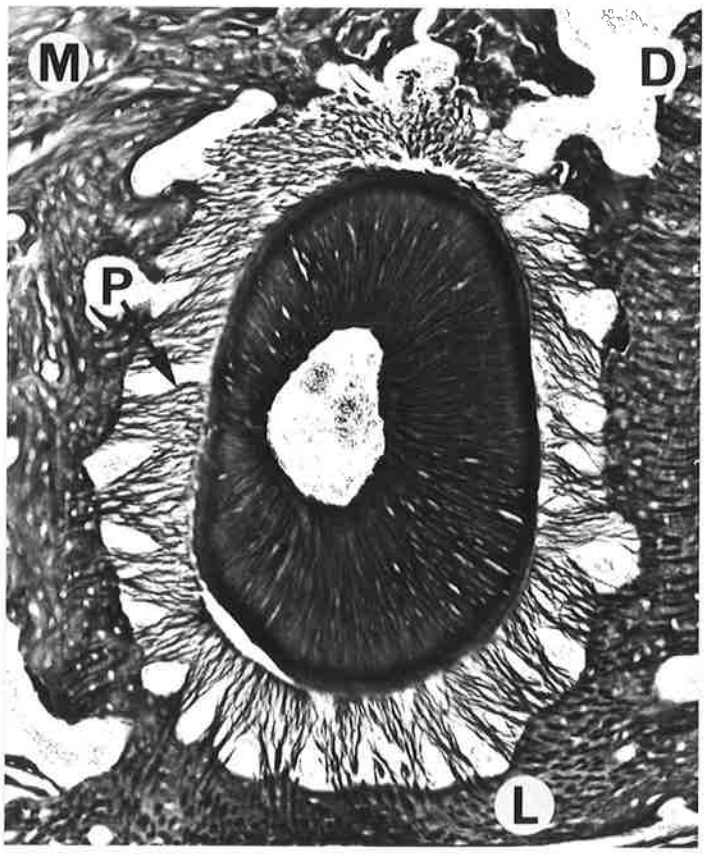


Figure 14. At the level of the mid-root principal fibres (P) are seen radiating from the distal root of the first molar in a 40-day control animal.
M, mesial; D, distal; L, lingual.

Herovici, x120.

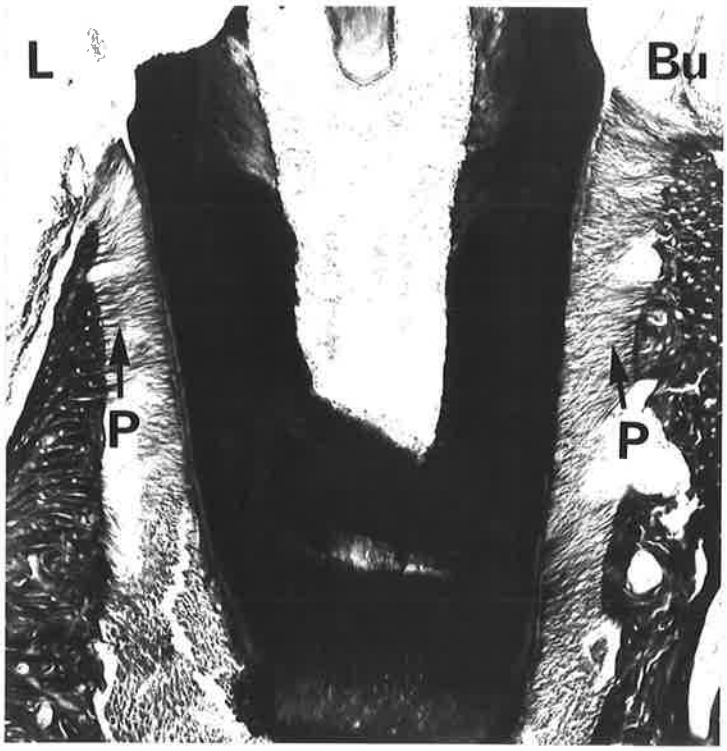


Figure 15. A buccolingual view of principal fibres (P) related to the mesial root of the second molar exhibiting size differences adjacent to buccal and lingual facing bone.
Bu, buccal; L, lingual.

Herovici, x100.

PRINCIPAL FIBRES.

Lathyritic Animals:

Lathyrism produced a disruption in the principal fibres which increased in severity to involve all subcrestal fibres as the experimental period proceeded (Fig. 16). During the early stages of the disease fibres seemed more loosely arranged, yet maintained their orientation. Although fibres appeared fewer in number (Fig. 17) no appreciable difference from normal fibre size was evident as they continued their wavy course from cementum to bone.

With the progression of lathyrism an examination of a large number of sections gave the impression of a decrease in width of the periodontal space. The accumulation of lathyritic material resulted in a disturbance of fibre orientation (Fig. 18). Fibre attachment to cementum and alveolar bone appeared extremely tenuous. Principal fibres which could be distinguished within the periodontal space were very fine and followed a haphazard course. Although minimal branching and intercommunication occurred there appeared a union of fewer fibres at both mesial and distal facing bone surfaces. In areas of greatest lathyritic change fibres were obscured by clumps of lathyritic material (Fig. 19). However, birefringence, under polarizing microscopy, revealed an internal fibrous structure of these amorphous masses (Fig. 20).

In horizontal section many principal fibres were seen in elliptical-section indicating a vertical component to their random arrangement (Fig. 21) possibly due to an induced lathyritic change in orientation.

Similar principal fibre disorganization was observed in the buccolingual plane.

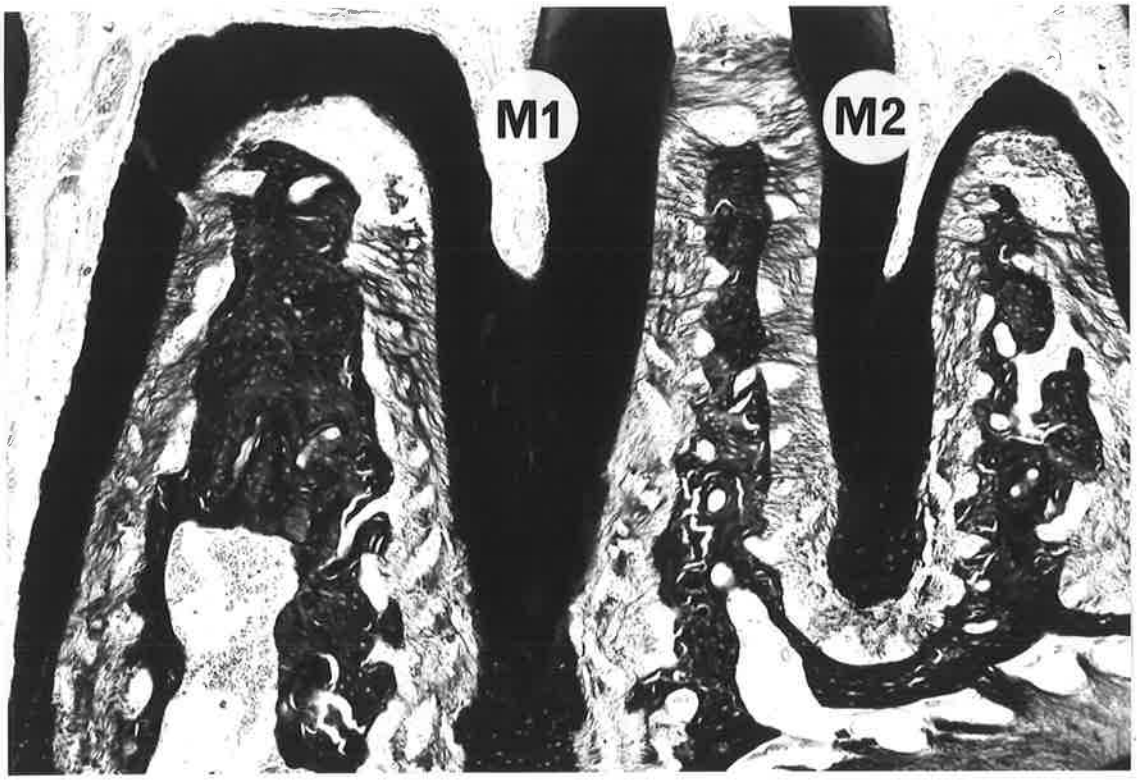


Figure 16. A mesiodistal section showing the widespread disruption of the periodontal ligament surrounding the first and second molars (M1, M2) of a 40-day lathyritic mouse.

Herovici, x65.

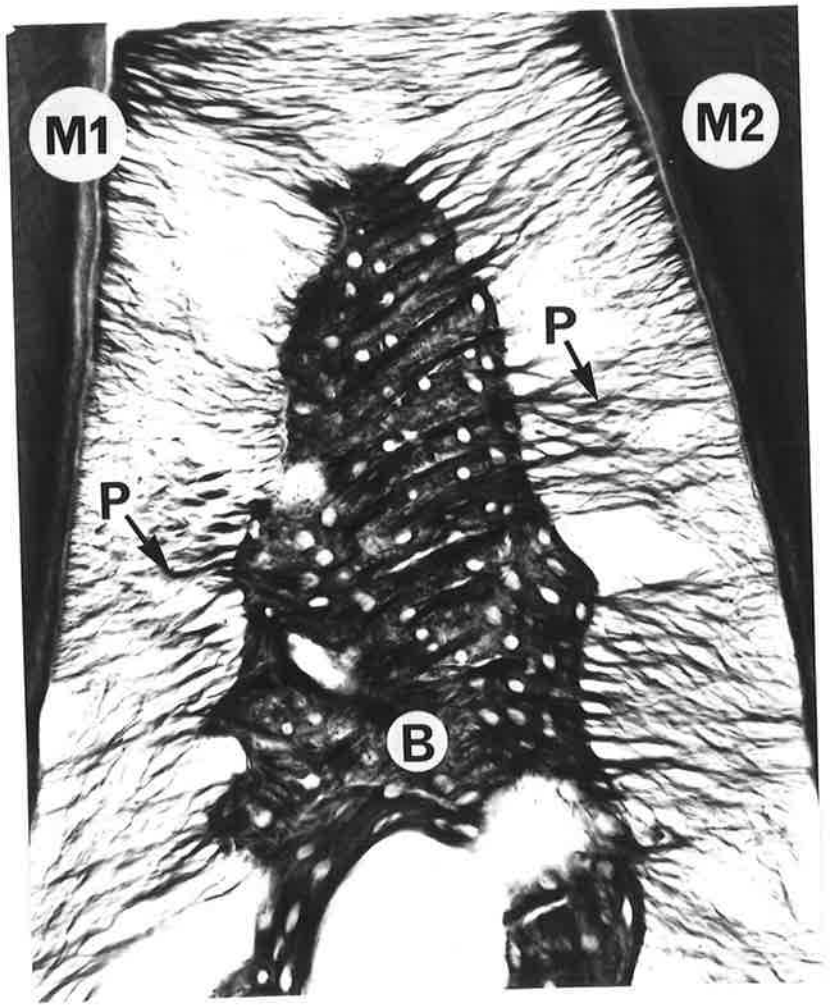


Figure 17. A mesiodistal section of principal fibres (P) between the first and second molars (M1, M2) and interdental septum (B) of a 10-day lathyritic animal. Principal fibres appear less numerous and more loosely arranged.

Herovici, x150.

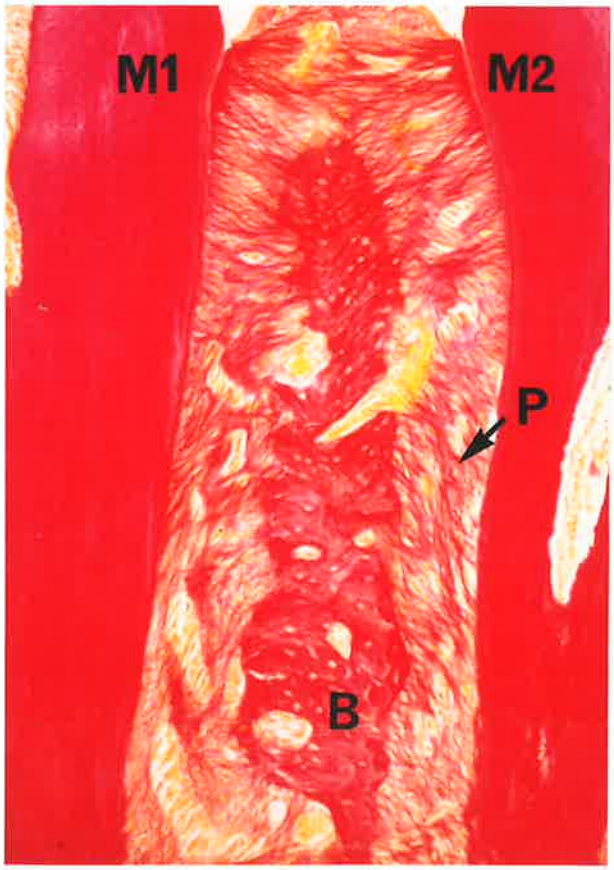


Figure 18. A mesiodistal section of the interdental septum (B) and first and second molars (M1, M2) showing the disturbance of principal fibres (B) after 40 days of lathyrism. (Compare with Fig. 11).

Herovici, x120.

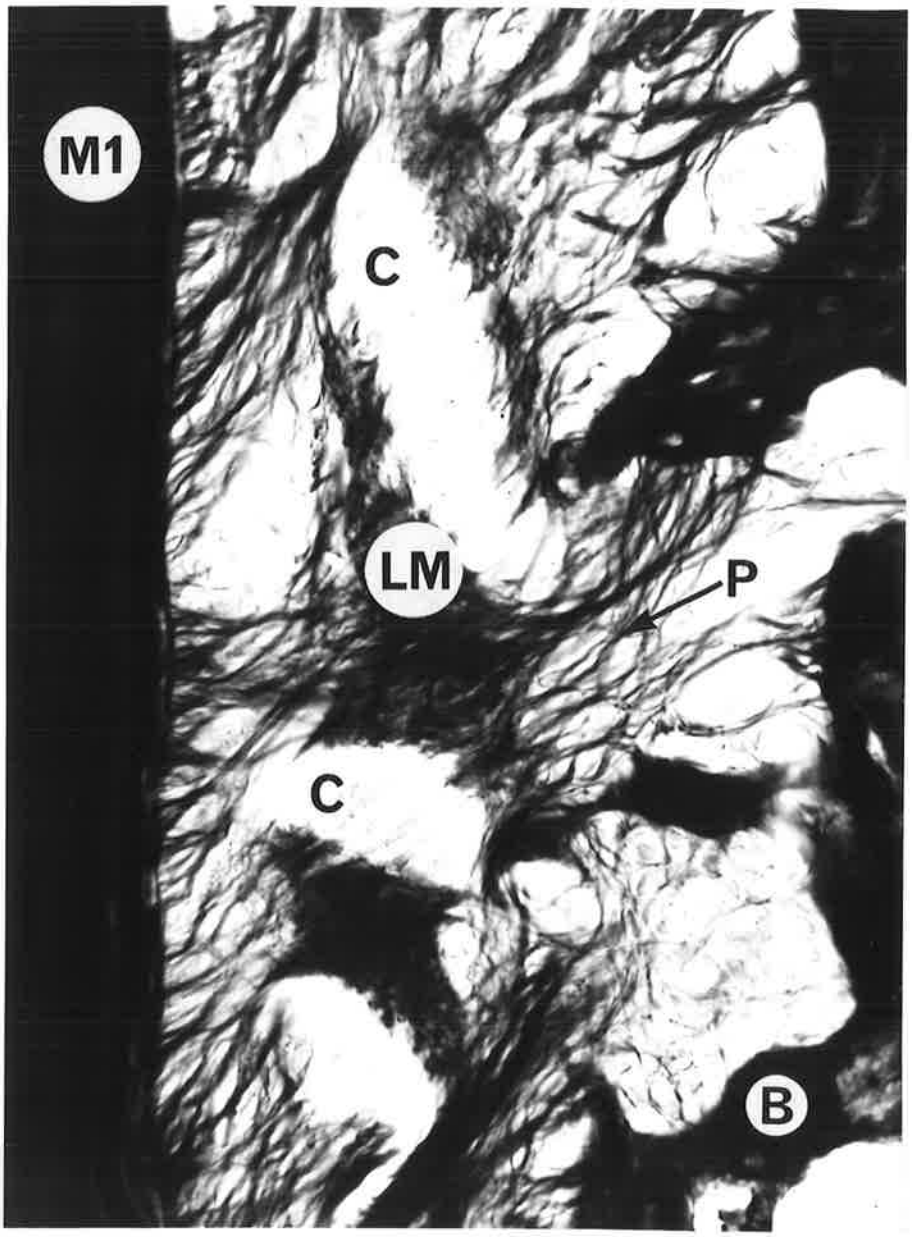


Figure 19. The periodontal ligament between the first molar (M1) and the mesial facing surface of the interdental septum (B) revealing fine principal fibres (P) often obscured by the lathyritic material (LM) and pallisading cells (C).

Herovici, x450.

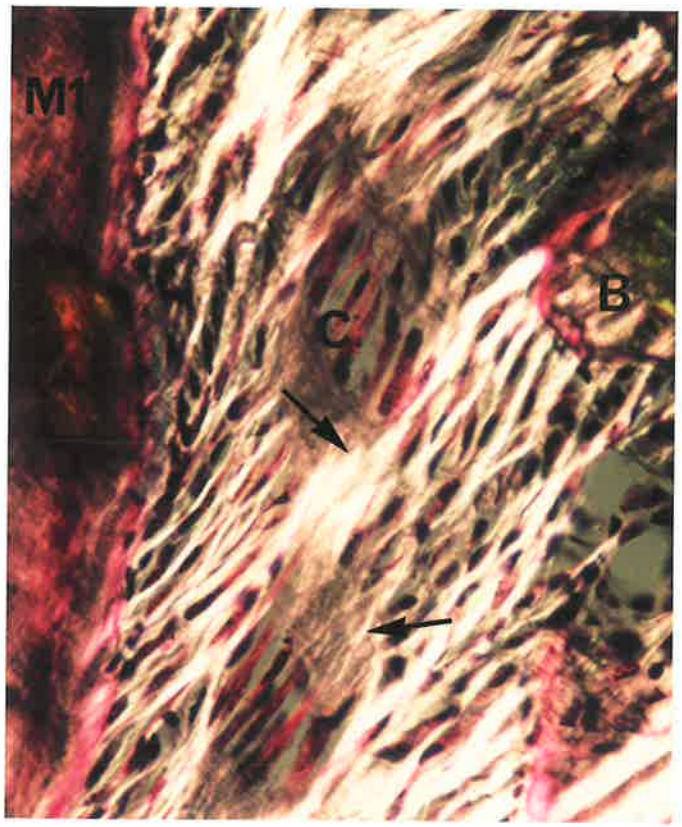


Figure 20. A mesiodistal section of a lathyritic lesion between the first molar (M1) and interdental septum (B) revealing birefringence (arrowed) suggesting a fibrous internal structure within the lathyritic material. C, pallisading cells.

H & E under polarized light, x450.

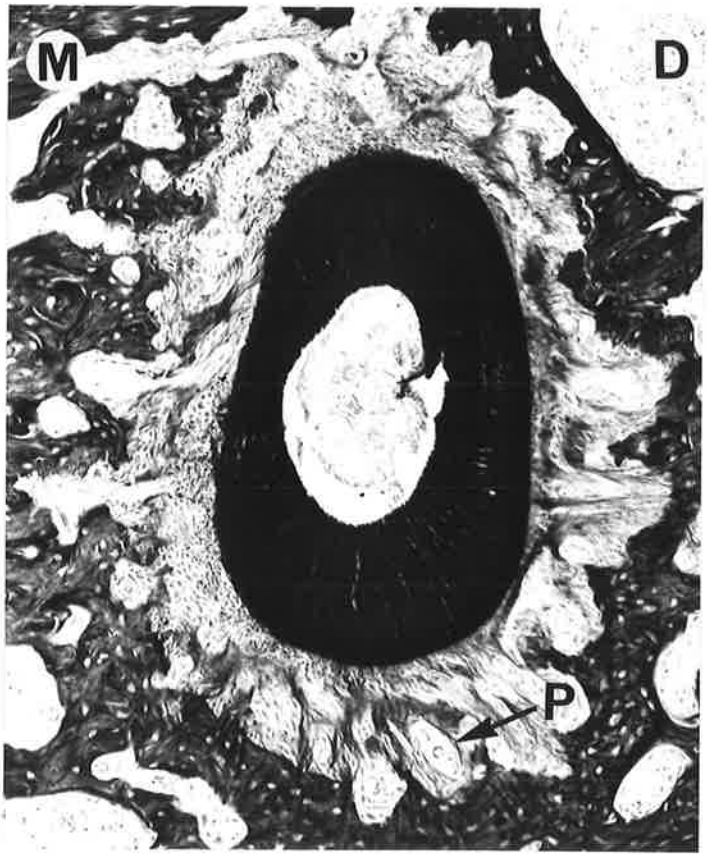


Figure 21. A horizontal section through the distal root of the first molar of a 40-day lathyritic mouse revealing the haphazard principal fibre (P) arrangement many of which are seen in elliptical-section. (Compare with Fig. 14).
M, mesial; D, distal.

Herovici, x120.

SHARPEY FIBRES.

Control Animals:

Sharpey fibres appeared as distinct structures embedded within bone (Fig. 22). Their formation was seen as the union of several principal fibres into a large fibre trunk. The size (diameter) of Sharpey fibres varied according to the number of uniting principal fibres. In mesiodistal and horizontal sections the distal facing surfaces of alveolar bone were perforated by large Sharpey fibres (Fig. 22, 23, 24, 25). This pattern appeared to correspond with the large size of the principal fibres of the adjacent periodontal ligaments.

The largest Sharpey fibres were found at the alveolar crest and extended into bone for great distances (Fig. 22). Fibres often reached the mesial facing bone surface, but were not seen to enter the opposite periodontal ligament. Further apically penetrating fibres that did not cross the entire width of the alveolar septa terminated at incremental lines within bone (Fig.23).

The mesiodistal orientation of these penetrating fibres changed slightly within the alveolar septa. At the alveolar crest Sharpey fibres maintained the direction of the alveolar crest principal fibres. They perforated bone in an oblique occlusoapical direction.

This orientation became more horizontal as the crestal Sharpey fibre group penetrated the septal bone further apically. In the apical two thirds of the interdental bone Sharpey fibres were seen to follow a curved path. After entering bone in an apico-occlusal direction in line with the orientation of the principal fibres, Sharpey fibres turned apically forming a pattern resembling an "inverted V" (Fig. 24).

Mesiodistal and horizontal sections revealed indistinct Sharpey fibre patterns at mesial facing bone surfaces (Figs. 22, 24, 25). Short penetration of small fine fibres was occasionally seen. However, in the main, principal fibres failed to penetrate those bone surfaces but merely attached to its surface.

Moreover, an examination of mesiodistal sections approximating the lingual aspect of the teeth revealed Sharpey fibres deeply penetrating bone through the mesial facing surfaces (Fig. 26). An "upright V" pattern was formed with fibres entering from the opposite surface, yet no continuity of these transosseous fibres could be seen (Fig. 26). The penetration of Sharpey fibres from the mesial facing surface gradually receded as more buccally located sections were examined. The penetration of Sharpey fibres into the lingual aspect of mesial facing bone surfaces and their receding nature could also be seen in horizontal sections (Fig. 25).

Principal and ey fibre continuity was also seen through buccal ; surfaces of bucco-lingual sections (Fig. 27

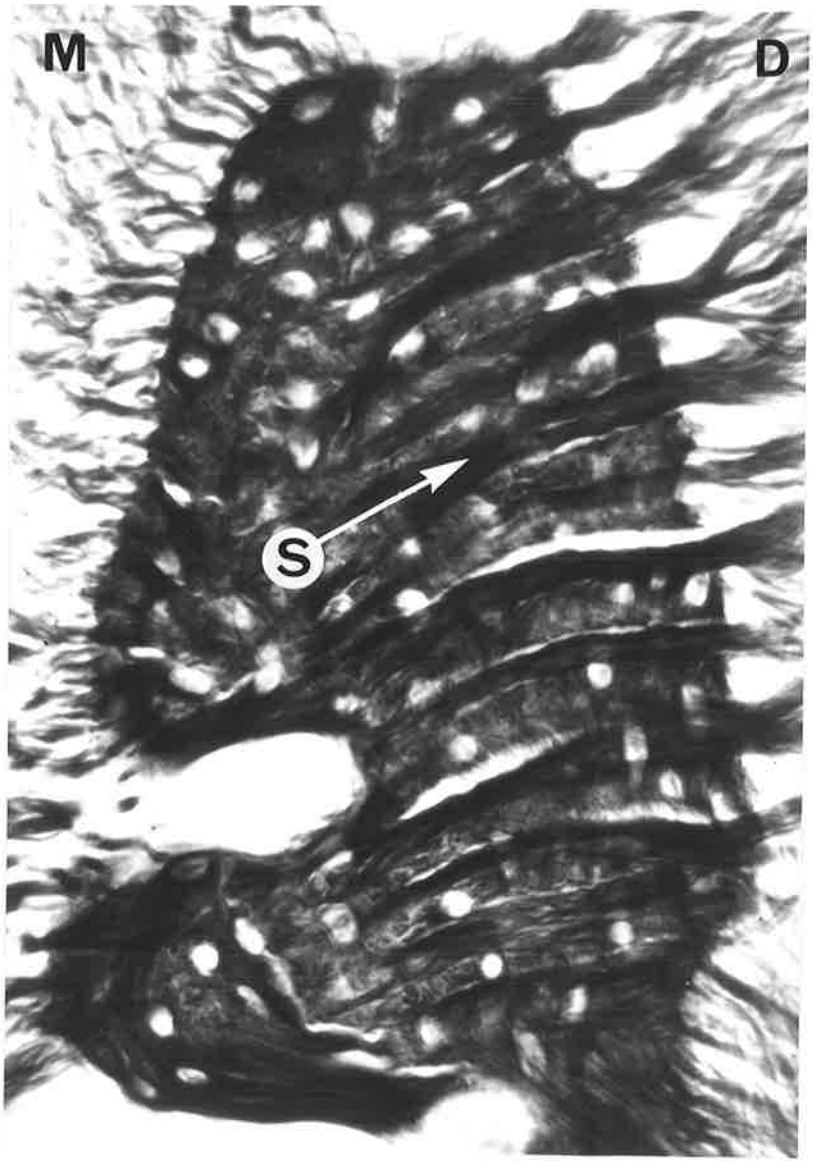


Figure 22. Sharpey fibres (S) within alveolar crest bone of a 40-day control animal. Their formation is seen as the union of several principal fibres with size variation at mesial and distal facing bone surfaces. M, mesial; D, distal.

Herovici, x400.

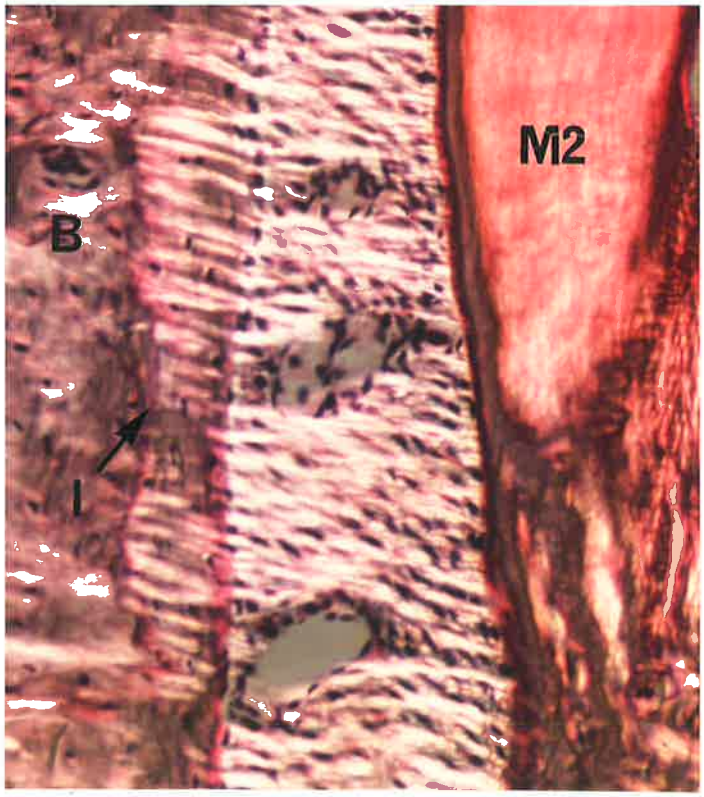


Figure 23. A mesiodistal section between the second molar (M2) and the interdental septum (B) showing birefringent principal fibres penetrating bone as Sharpey fibres and terminating at an incremental line (I).

H & E under polarized light, x400.

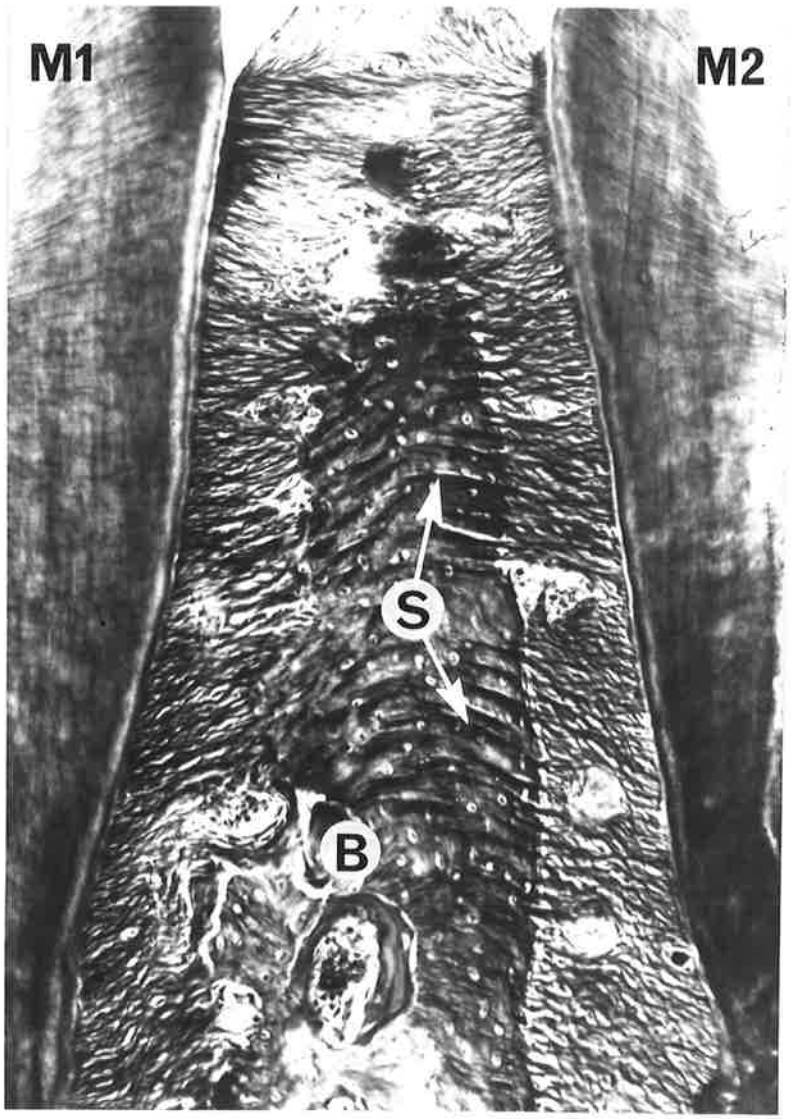


Figure 24. Sharpey fibres (S) within the interdental septum (B) between the first and second molars (M1, M2) of a 40-day control mouse showing their curved pathway resembling an "inverted V" within bone.

Cason's stain, x120.

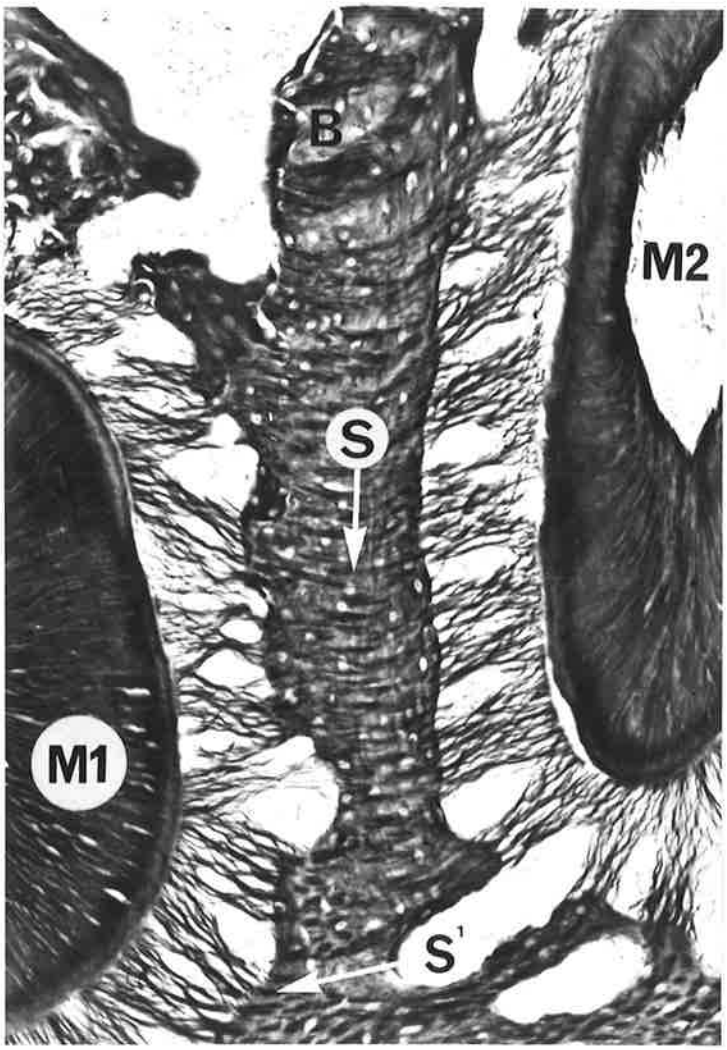


Figure 25. A horizontal section showing Sharpey fibres (S) penetrating the distal facing surface and passing undeviated through alveolar bone (B). Sharpey fibre penetrations (S^1) of mesial facing bone is seen on the distolingual aspect of the first molar (M1).
M2, second molar.

Herovici, x150.

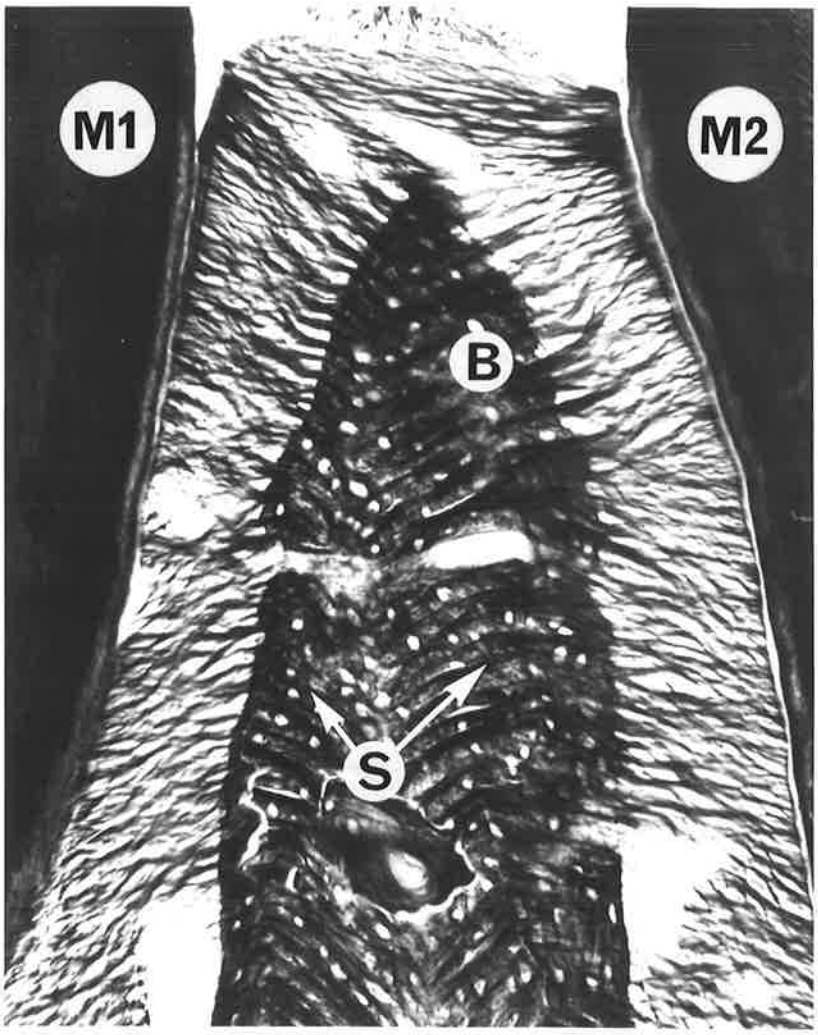


Figure 26. A mesiodistal section of the interdental septum (B) between the first and second molars (M1, M2) cut close to the lingual aspect of the teeth revealing Sharpey fibres (S) penetrating both mesial and distal facing surfaces and forming an "upright V" pattern within bone.

Herovici, x150.

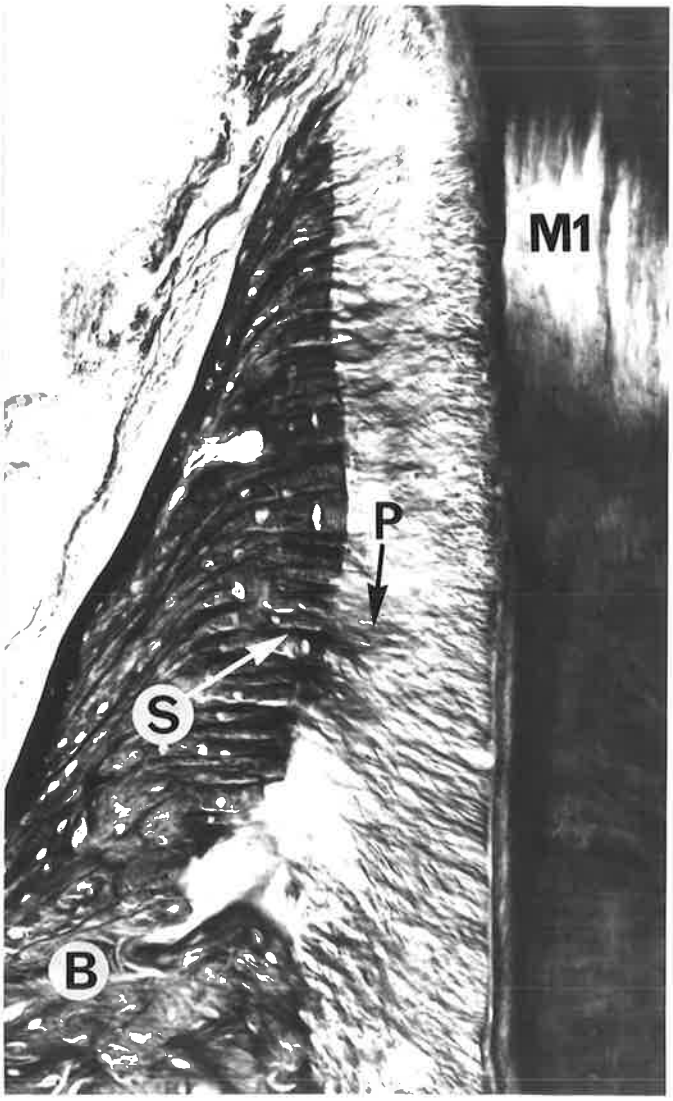


Figure 27. A buccolingual section through the distal root of the first molar (M1) of a 40-day control mouse showing continuation of principal fibres (P) through the buccal facing surface of lingual alveolar bone (B) as Sharpey fibres (S).

Herovici, x200.

SHARPEY FIBRES.

Lathyritic Animals:

Once embedded in bone the morphology of Sharpey fibres was not seen to change unless bone was resorbed. Compared with control animals the intra-bony orientation of Sharpey fibres in lathyritic mice appeared to be maintained (Fig. 28). However, at distal facing surfaces and also mesial facing surfaces in the case of lingually located sections the continuity of principal fibres into bone as Sharpey fibres became progressively disturbed.

The penetration of Sharpey fibres into bone was maintained at the crests of the interdental and interradicular septa (Fig. 28). The lathyritic disturbance of the periodontal ligament appeared associated with a discontinuity of principal and Sharpey fibres at the bony interface (Fig. 28). Sharpey fibres terminated at mesial and distal facing surfaces which were rough and irregular. The irregular bone surfaces plus the discontinuity of principal and Sharpey fibres appeared consistent with resorptive bone surfaces and therefore a change in alveolar bone activity. In particular the distal facing bone surface, originally depository in nature, was, after forty days of lathyrism, mostly resorptive in character.

In horizontal (Fig. 29) and buccolingual (Fig. 30) sections fibre discontinuity was apparent around the entire root except at the alveolar crest.

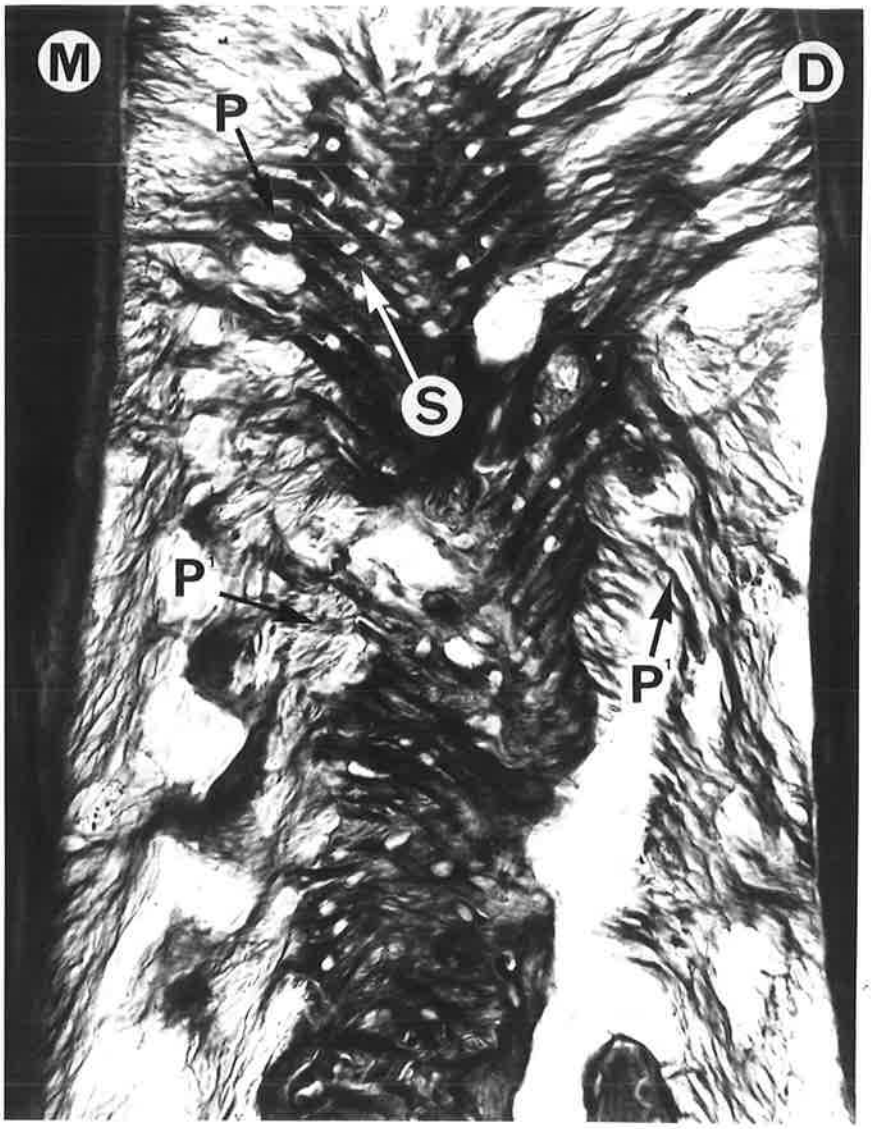


Figure 28. A mesiodistal section through the interdental septum close to the lingual aspect of a 40-day lathyritic mouse. The "upright V" pattern of Sharpey fibres (S) is maintained. Principal fibres (P) are continuous into bone as Sharpey fibres at the alveolar crest. Opposite areas of lathyritic periodontal ligament disturbance a discontinuity between principal fibres (P^1) and Sharpey fibres is seen. In these areas Sharpey fibres appear to terminate at rough and irregular bone surfaces. (Compare with Fig. 26).

M, mesial; D, distal.

Herovici, x200.

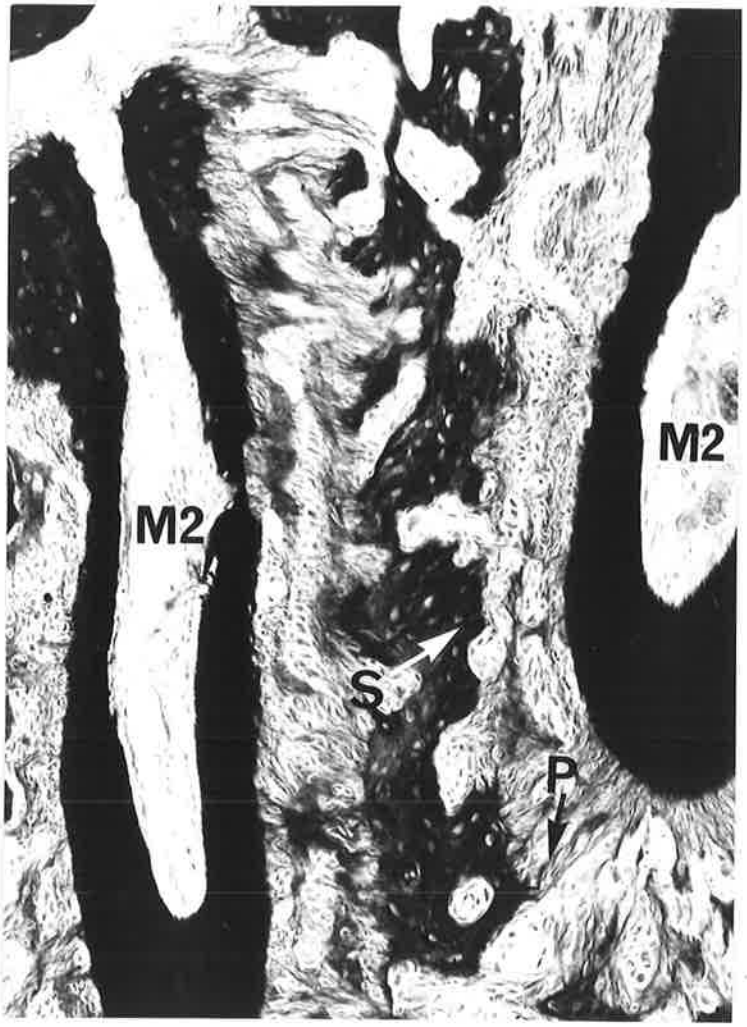


Figure 29. A horizontal section of the interradicular area of the second molar (M2) revealing the Sharpey fibre (S) and principal fibre (P) discontinuity.

Herovici, x150.

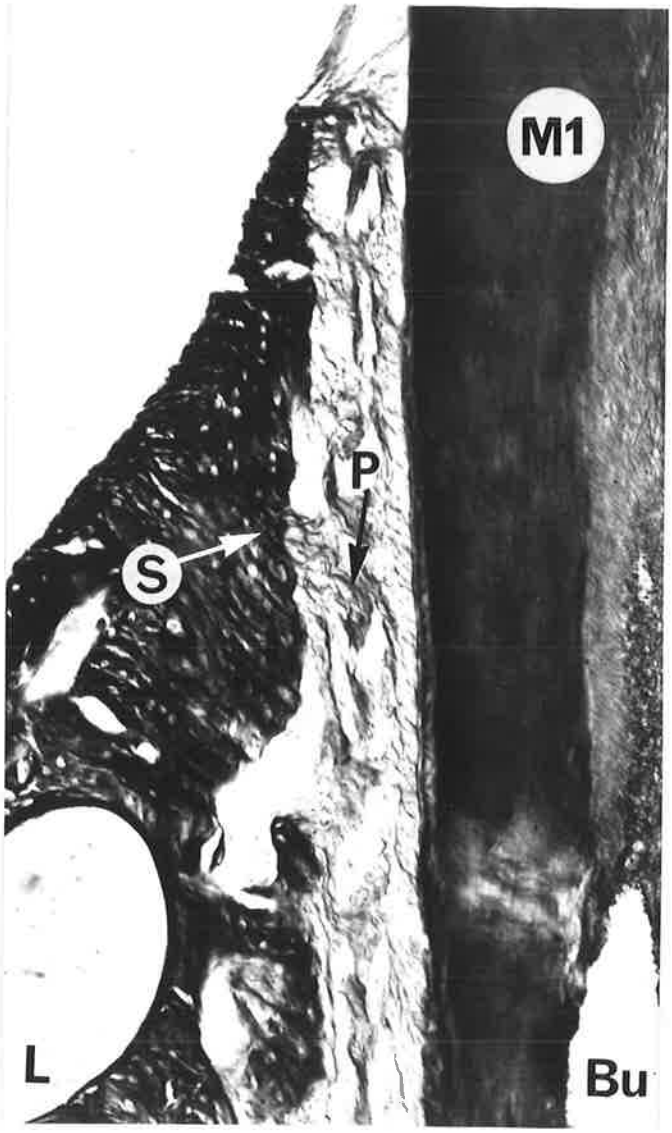


Figure 30. A buccolingual section through the first molar (M1) of a 40-day lathyritic mouse showing the discontinuity of Sharpey (S) and principal (P) fibres at the buccal facing bone surface. (Compare with Fig. 27). Bu, buccal; L, lingual.

Herovici, x200.

ALVEOLAR BONE.

Control Animals:

Interdental and interradicular alveolar bone contained osteocytes, a few trabecular spaces and exhibited a compact appearance characteristic of woven bone (Fig. 31).

The bony margins of the alveolus presented two varying pictures. There were regions where the surface of bone was smooth yet punctured by the insertion of Sharpey fibres (Figs. 31, 32). Fibre penetration was evident to a varying extent and produced bundle or Sharpey fibre bone. Within bone and running parallel to the smooth surface were regularly spaced, darkly staining incremental lines where Sharpey fibres were often seen to terminate (Figs. 23, 32). A single layer of osteoblast-like cells lined the surface and appeared characteristic of smooth-surfaced depository bone (Figs. 31, 32). In mesiodistal sections such depository bone sites were generally identified at the alveolar crest and at distal facing surfaces.

Elsewhere alveolar bone surfaces presented a rough irregular outline (Fig. 33). Sharpey fibre insertions were tenuous or non-existent. Principal fibres merely made surface contact and appeared maintained by spot deposition of bone. Alternating along these irregular surfaces were resorption lacunae with the

occasional osteoclast, reversal foci, bone formation foci and areas of rest or quiescence (Fig. 33).

The smooth and rough bony outlines, varying respectively with the insertion of Sharpey fibres, were apparent in horizontal sections (Fig. 34). The irregular outline was seen to face mesially and also lingually. A smooth outline with accompanying osteoblast-like cells was seen on the distal and buccal facing bone surfaces.

Buccolingually, the smooth nature of the buccal facing, and the irregular nature of the lingual facing bone surfaces, was confirmed.

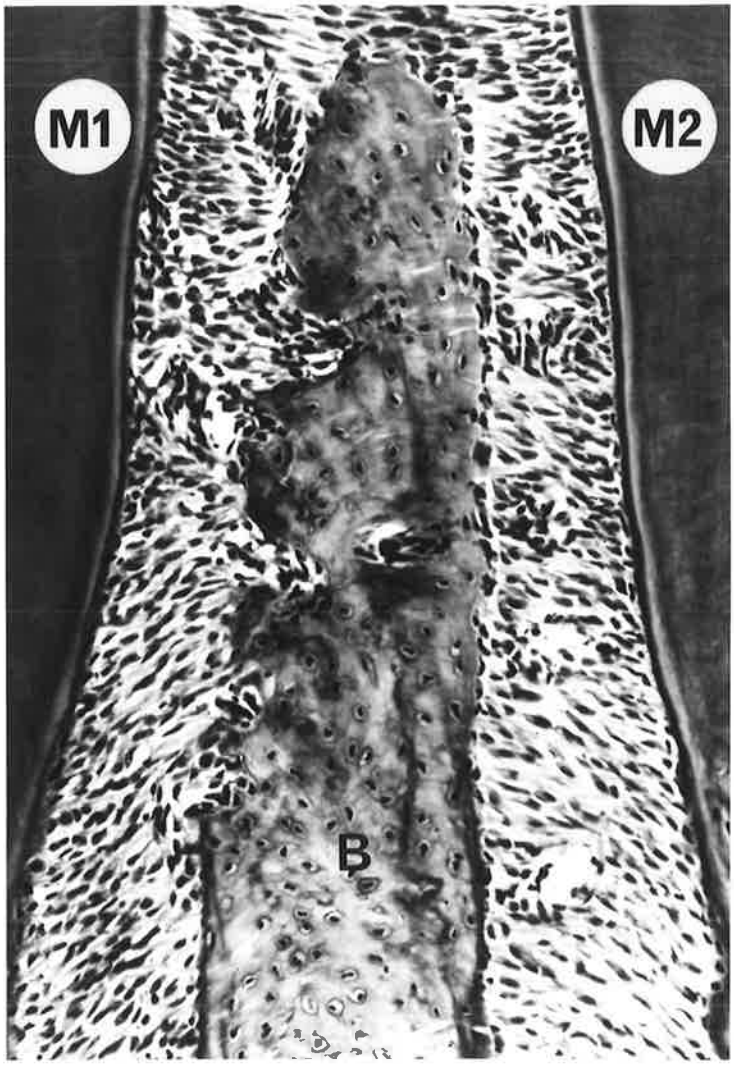


Figure 31. The interdental septum (B) between the first and second molars (M1, M2) of a 40-day control animal exhibits a compact appearance. The distal facing surface lined by a single layer of cells presents a smooth bone margin, while a more irregular margin comprises the mesial facing surface.

Moss and Gray, x150.

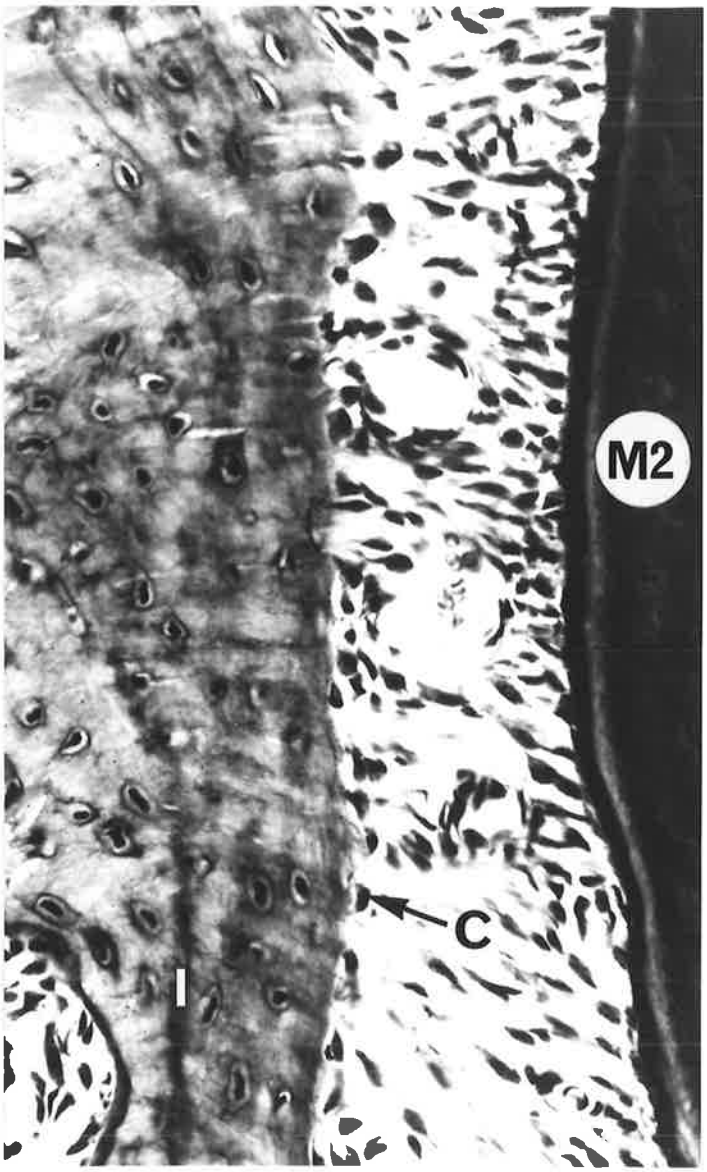


Figure 32. A depository bone surface having a smooth margin with penetrating fibres and single layer of surface cells (C) faces the second molar (M2) of a 40-day control mouse. An incremental line (I) can be seen within the bone.

Moss and Gray, x450.

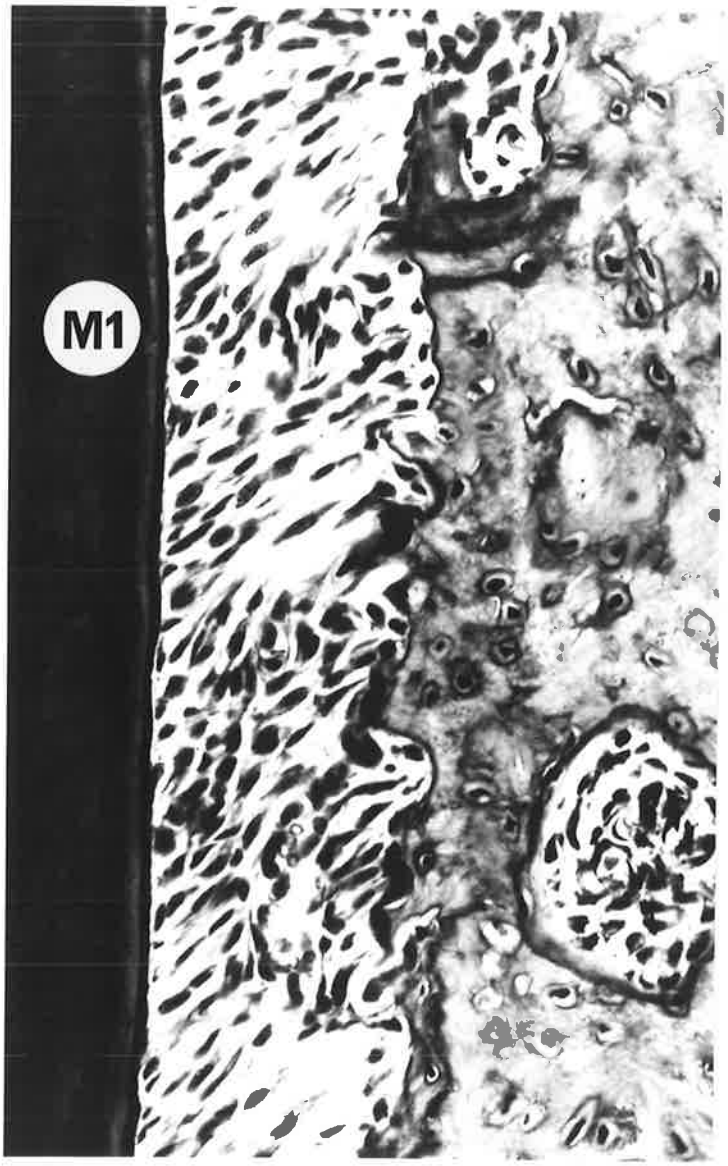


Figure 33. A resorptive surface having an irregular bony outline faces the first molar (M1) of a 40-day control mouse.

Moss and Gray, x450.

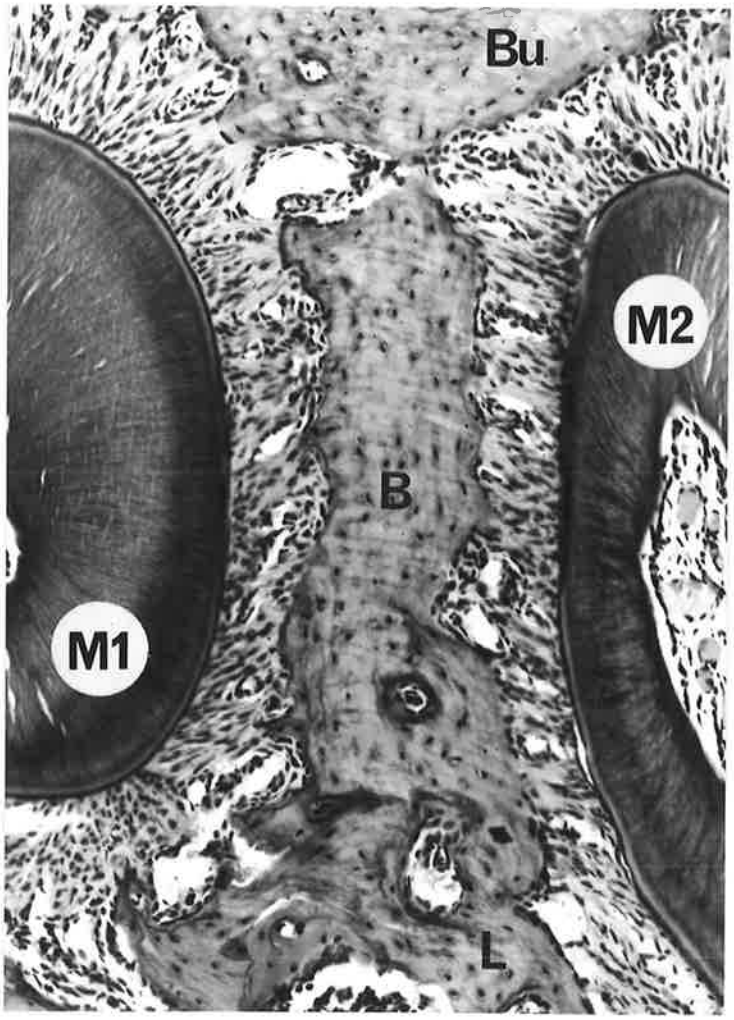


Figure 34. A mid-root horizontal section through the interdental septum (B) between the first and second molars (M1, M2) of a control animal showing the compact nature and surface outline of bone.
Bu, buccal; L, lingual.

H & E, x150.

ALVEOLAR BONE.

Lathyritic Animals:

The architecture of alveolar bone in the lathyritic mouse was greatly disturbed by an increase in trabeculation. Bone in the interdental regions appeared extremely porous and less dense in comparison with normal animals (Fig. 35). The bony outline was grossly irregular (Figs. 35, 36). Surface regularity was destroyed by massive resorption areas and the presence of blood vessels. The distal facing surfaces of the alveolar septa changed from smooth to irregular surface outlines apparently in association with the lathyritic disorganization of the periodontal ligament. Bone resorption appeared to accompany the development of the lathyritic lesion. However, smooth surfaces were maintained near the alveolar crest where the lathyritic change in the periodontal ligament was less severe.

Horizontal (Fig. 36) and buccolingual sections showed the extent of the bone porosity to be widespread. Large blood-filled trabecular spaces decreased bone density over the entire width of the mandible. The irregularity of the bone outline was evident on both buccal and lingual facing surfaces in association with the lathyritic change.

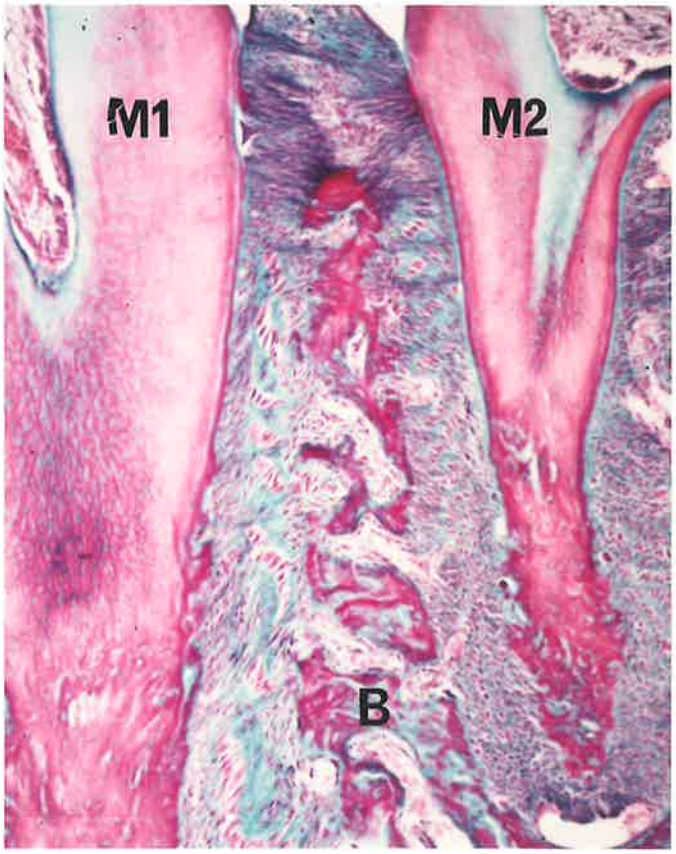


Figure 35. Bone changes after forty days of lathyrism. Severe porosity and an irregular bony outline of the interdental septum (B) is evident between the first molar (M1) and second molar (M2).

Pollack's trichrome, x120.

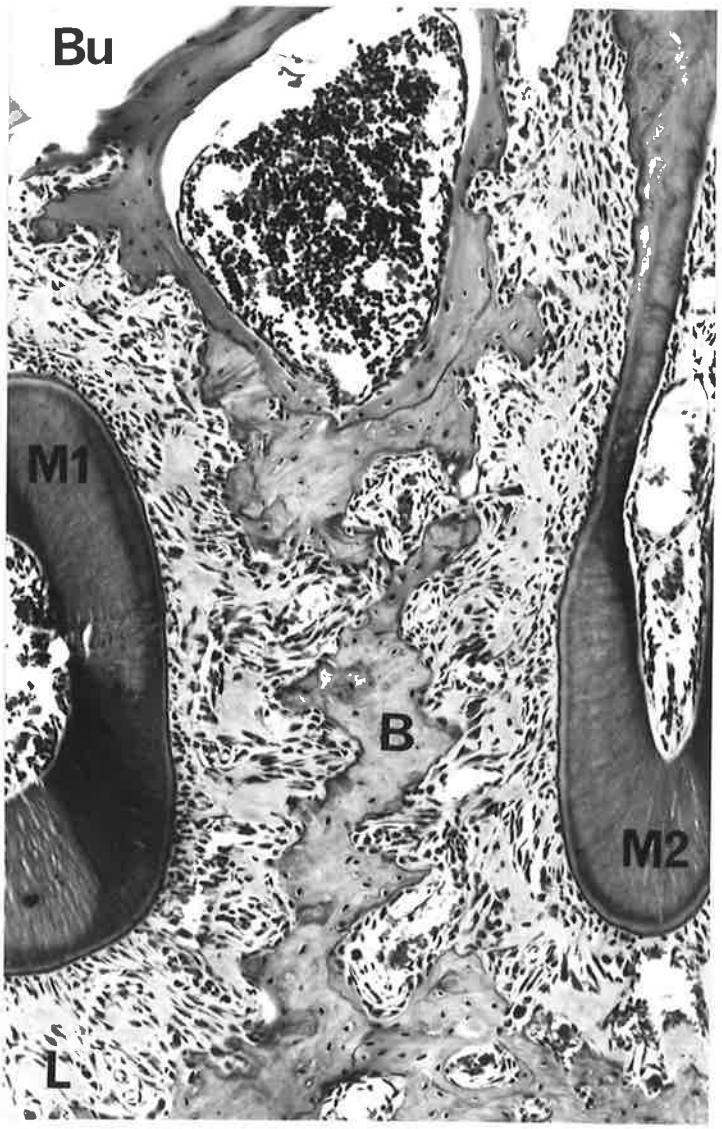


Figure 36. A horizontal section through the interdental septum (B) between the first and second molars (M1, M2) of a 40-day experimental animal showing the irregularity of the bony outline. (Compare with Fig. 34).

Bu, buccal; L, lingual.

H & E, x120.

THE MOUSE INCISOR.

Control Animals:

The incisor periodontal ligament differed compared with the molar ligaments. Three distinct cellular layers were apparent (Fig. 37). Two layers of spindle-shaped cells were associated with the cemental and alveolar surfaces, while a central layer of more rounded cells separated the two.

Principal fibres of the incisor periodontal ligament appeared finer, yet more densely arranged, than fibres of the molar ligaments. Intercommunication between adjacent fibres was frequent as incisor principal fibres coursed between cementum and bone.

Silver grain distribution within the incisor periodontal ligament reflected a high utilization of proline (Fig. 37).

THE MOUSE INCISOR.

Lathyritic Animals:

The effects of lathyrism seen in the molar periodontal ligament were not seen in the incisor periodontal ligament. The three cellular layers maintained their orientation without the appearance of the palli-sading effect (Fig. 38). Fibres continued to connect

cementum with bone in their wavy path.

The distribution of silver grains on radioautographs indicated a continuance of proline turnover (Fig. 38).

Figure 37.

A mesiodistal radio-autograph through the incisor (Ir) of a 40-day control animal shows the layered cellular arrangement and a dense distribution of silver grains within the periodontal ligament.
B, alveolar bone.

H & E, x200.

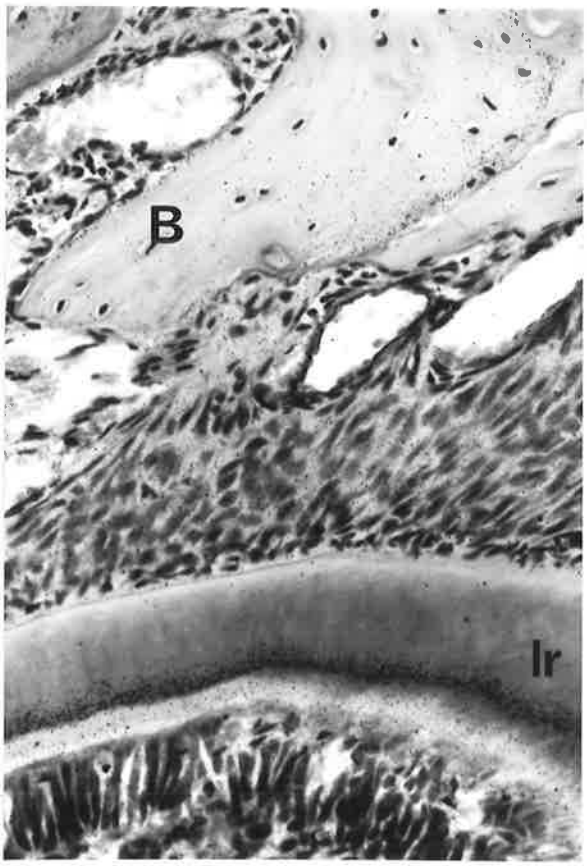
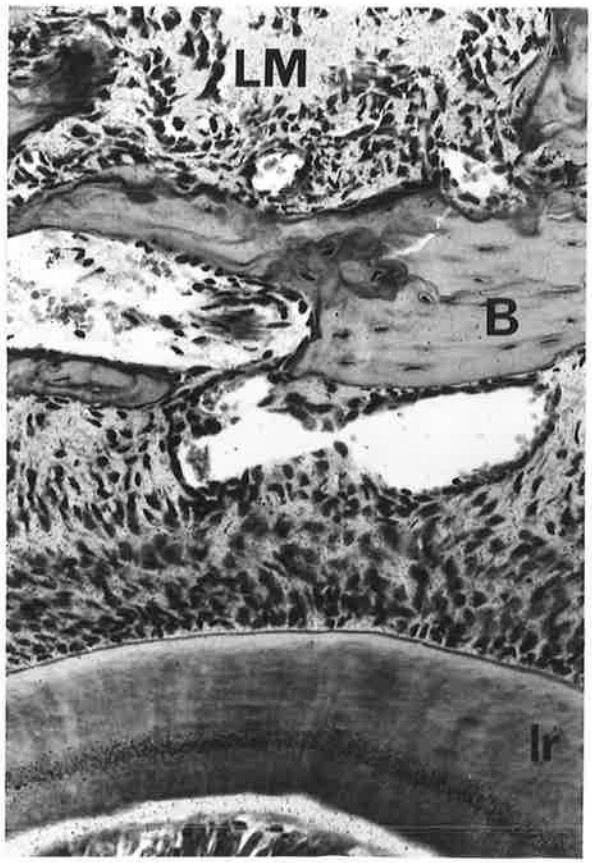


Figure 38.

A mesiodistal radio-autograph through the incisor (Ir) of a 40-day lathyritic mouse showing the maintenance of periodontal cell arrangement and a dense silver grain distribution indicates a continuing high turnover of proline. Lathyritic material (LM) may be seen in the molar periodontal ligament.
B, alveolar bone.

H & E, x200.



II. RADIOAUTOGRAPHY.

Silver grains were topographically distributed over radioautographs at sites where proline had been incorporated into tissues. Although no quantitative studies were performed, it appeared that silver grain concentration was less when Cason's technique (Cason, 1950) and Ehrlich's haematoxylin and eosin (Luna, 1968, p.35) were used relative to the technique of Mortreuil-Langlois (1962).

Silver grains were located in a gelatin layer over the periodontal ligament, dental pulp, dentine, cementum and alveolar bone. Control radioautographs revealed a low background level of radiation which was deemed to have an insignificant effect on the topographical distribution of silver grains. In turn, each area of the periodontium is discussed with the main emphasis being placed on silver grain patterns over alveolar bone.

CONTROL ANIMALS.

Periodontal Ligament:

A dense concentration of silver grains over the periodontal ligament of all control mice was associated with the injection of tritiated proline twenty-four hours prior to sacrifice (Fig. 39). Grains were found over the extracellular areas rather than over cells. The density

of silver grains appeared less over the supracrestal and interradicular crestal areas of the periodontal ligaments although no obvious gradation could be seen across the width of the periodontal space.

Dental Pulp, Dentine and Cementum:

A moderate concentration of silver grains over the dental pulp suggested reduced proline activity in this area compared with the periodontal ligament (Fig. 39). Grain location was once again seen to be extracellular. Lines of silver grains coinciding with the first and second administrations of proline were found within dentine surrounding the pulp. These lines indicated sites of odontoblastic pre-dentine formation. The distance separating the two lines increased as the experimental period proceeded, but always the site of silver grain line corresponding to the second dose of proline was at the pulp-dentine interface.

The lines of silver grains within dentine were continuous through cellular cementum at the root apex (Fig. 47). These cemental grain lines followed the contour of the root apex but did not extend further than its lateral margin. The distance between cemental grain lines was greater than for dentine and indicated a higher rate of cementum formation compared with dentine. Silver grains were neither seen in areas of acellular cementum lining the tooth root nor in the deeper dentine layers.

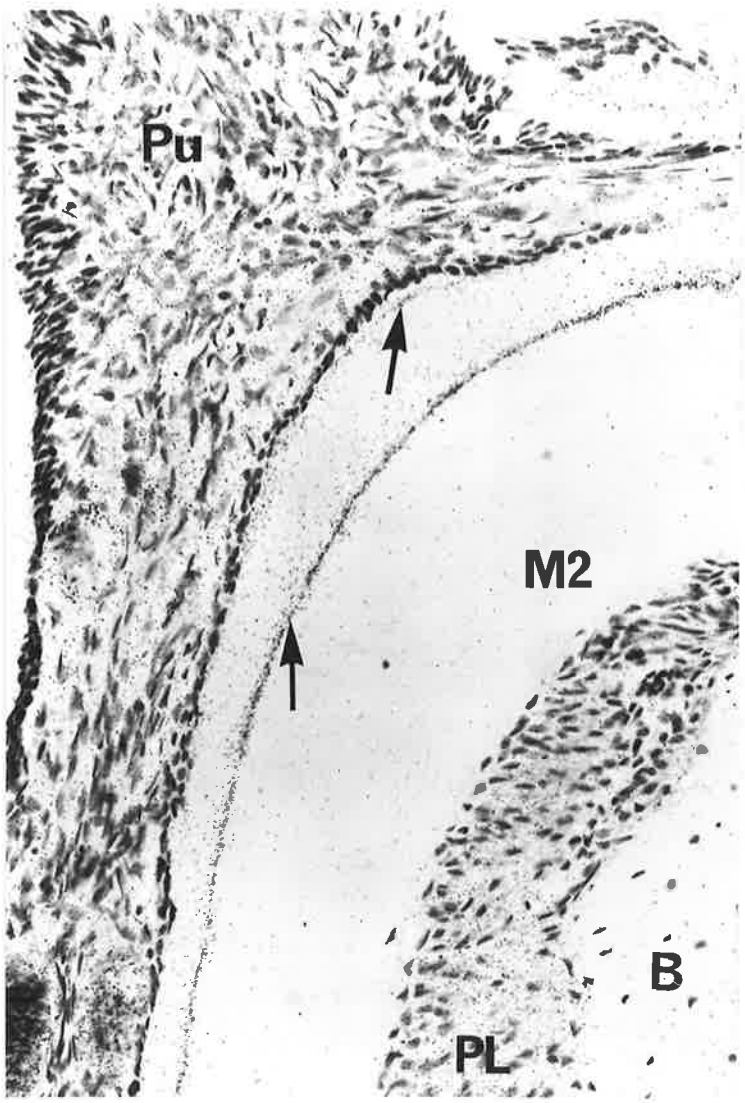


Figure 39. Silver grain distribution over the periodontal ligament (PL) and pulp (Pu) of control mouse. Two lines of silver grains (arrowed) are seen within dentine. M2, second molar; B, interradicular bone.

Nuclear fast red, indigo-carmine, x350.

Alveolar Bone:

The gross distribution of radioautographic bone label is presented in Figure 40. Two lines of silver grains, corresponding to the two administrations of tritiated proline, were seen over alveolar bone surrounding the mouse molars. In the 10-day control animal, the first injection of proline produced a line of silver grains that followed the contour of the alveolar crest and the distal facing bone surface to the apical region where it surrounded the root tip (Fig. 41). This line of label was maintained in all mesiodistal control sections (Figs. 41, 42). The demarcation of bone activity at the time of animal sacrifice was made using the second proline administration. Depository surfaces were revealed by silver grains at the bone-periodontal ligament interface (Fig. 43), whereas resorptive bone contained no surface label (Fig. 44) except in isolated areas where spot deposition appeared to maintain ligament fibre attachment.

The final injection of proline producing the second line of label indicated continuing bone deposition at the alveolar crest, along the distal facing surface and at the socket apex in all control animals (Figs. 41, 42). The distance between the two lines of silver grains increased progressively with the length of the experimental period (Fig. 42). Bone deposition at the alveolar crest exceeded that at the distal facing surface. Bone deposition at the alveolar crest also exceeded that in the apical region. However, the discrepancy appeared to

be compensated for by the laying down of cementum at the root apex.

Occasionally silver grain deposition was seen along the length of the mesial facing surfaces of the alveolar bone (Fig. 45). This was found in radioautographs sectioned and examined close to the lingual aspect of the teeth.

In horizontal sections (Fig. 46) the lines of silver grains encircled the mesial and lingual aspects of the teeth. These encircling lines of label, therefore, reflected bone deposition of distal and buccal facing bone surfaces (Fig. 46).

Buccolingually, silver grains were seen at both buccal and lingual alveolar crests and along the buccal facing bone surface to the apical region (Fig. 47).

The area between the two lines of label in all planes of section contained a collection of silver grains which were more densely gathered adjacent to the first grain line (Fig. 48). These markers appeared to lie over embedded Sharpey fibres which at the time of the first proline administration were principal fibres of the periodontal ligament.

Apart from label over osteocytes and minimal background levels there were few silver grains over other areas of bone matrix.

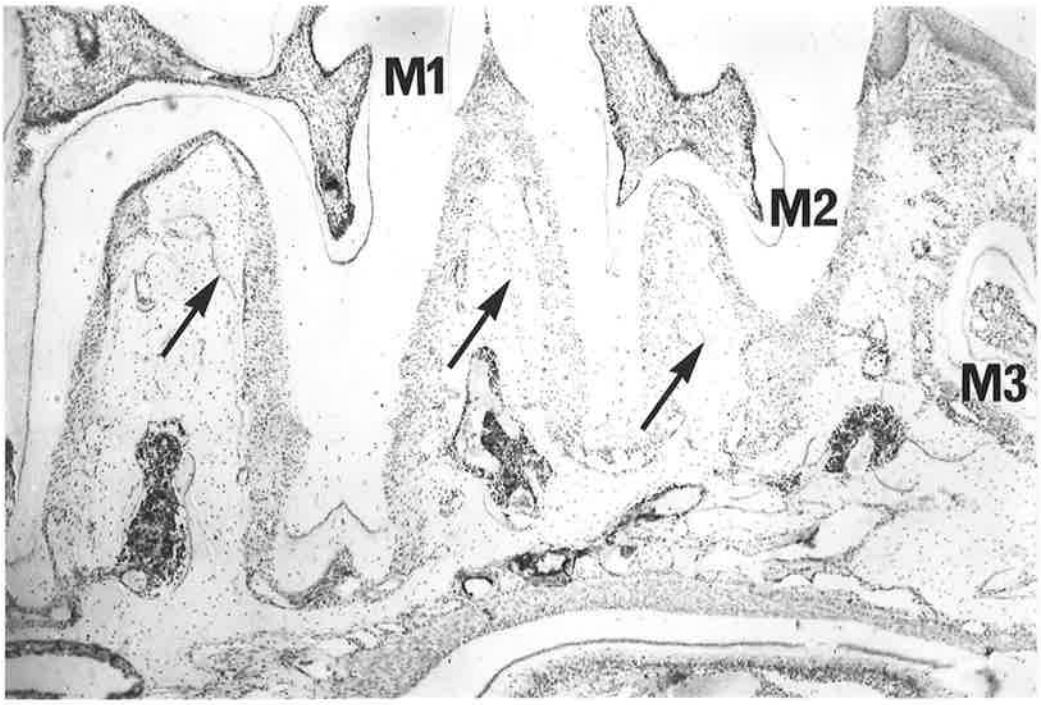


Figure 40. A mesiodistal radioautograph of the three molars (M, M2, M3) of a 40-day control mouse showing the gross distribution of silver grains (arrowed) within bone.

Nuclear fast red, indigo-carmine, x60.

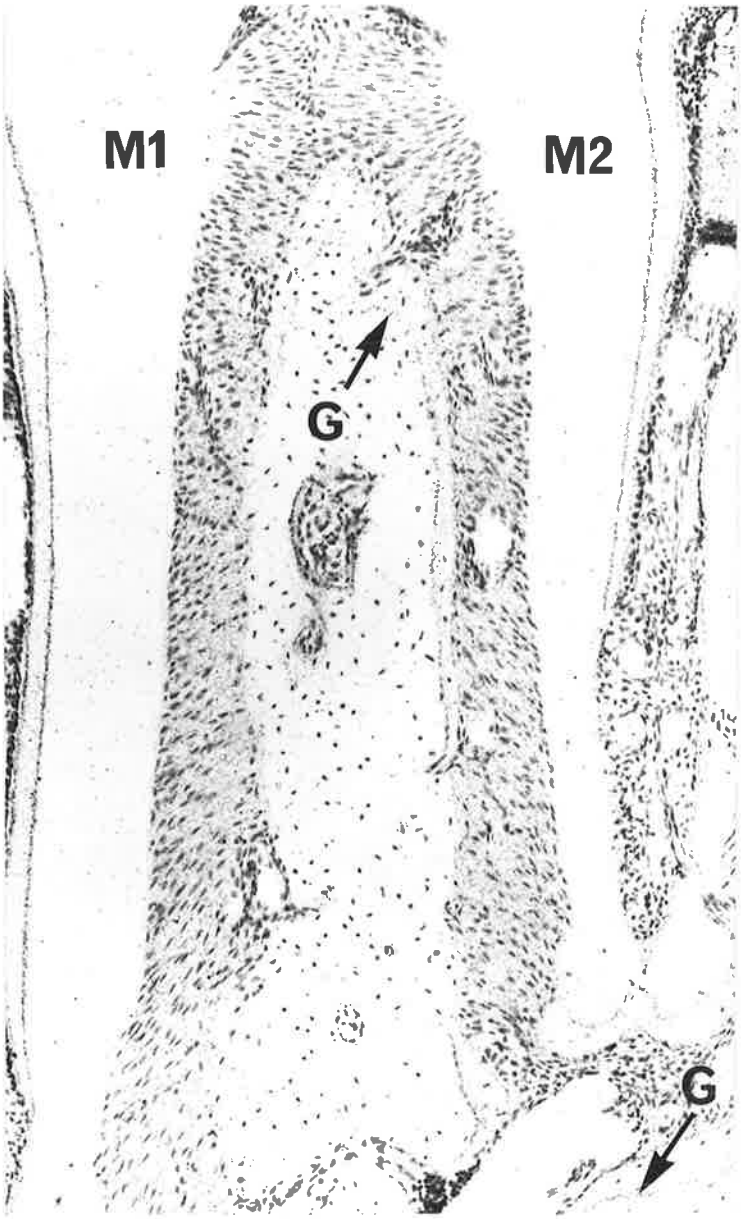


Figure 41. A mesiodistal radioautograph between the first and second molars (M1,M2) of a 10-day control mouse revealing silver grains (G) at the alveolar crest, the distal facing surface of the interdental septum and around the root apex of M2.

Nuclear fast red, indigo-carmine, x120.

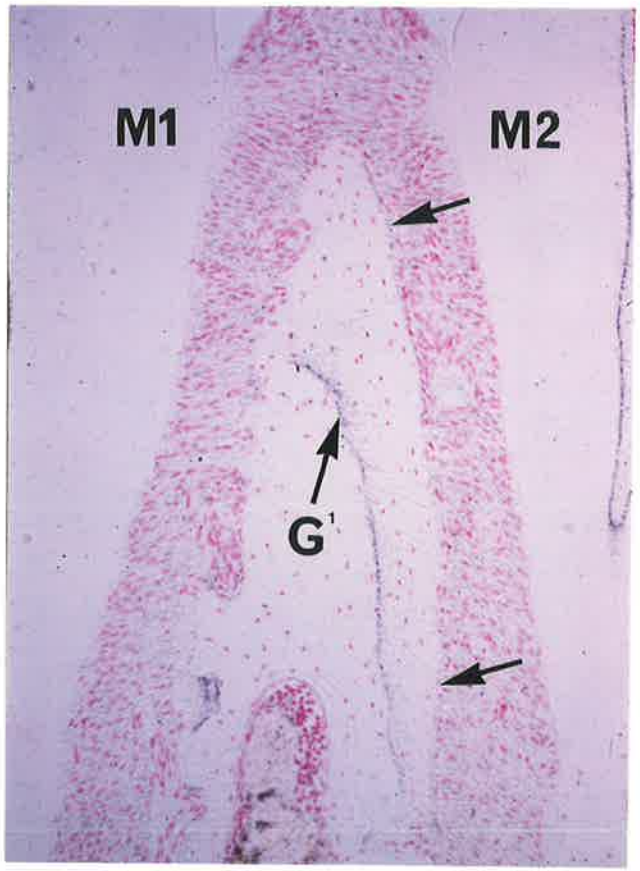


Figure 42. A mesiodistal radioautograph of the first and second molars (M1, M2) of a 40-day control mouse showing continuing bone deposition (arrowed) at the alveolar crest and distal facing bone surface. G^1 - initial silver grain line.

Nuclear fast red, indigo-carmine, x120.

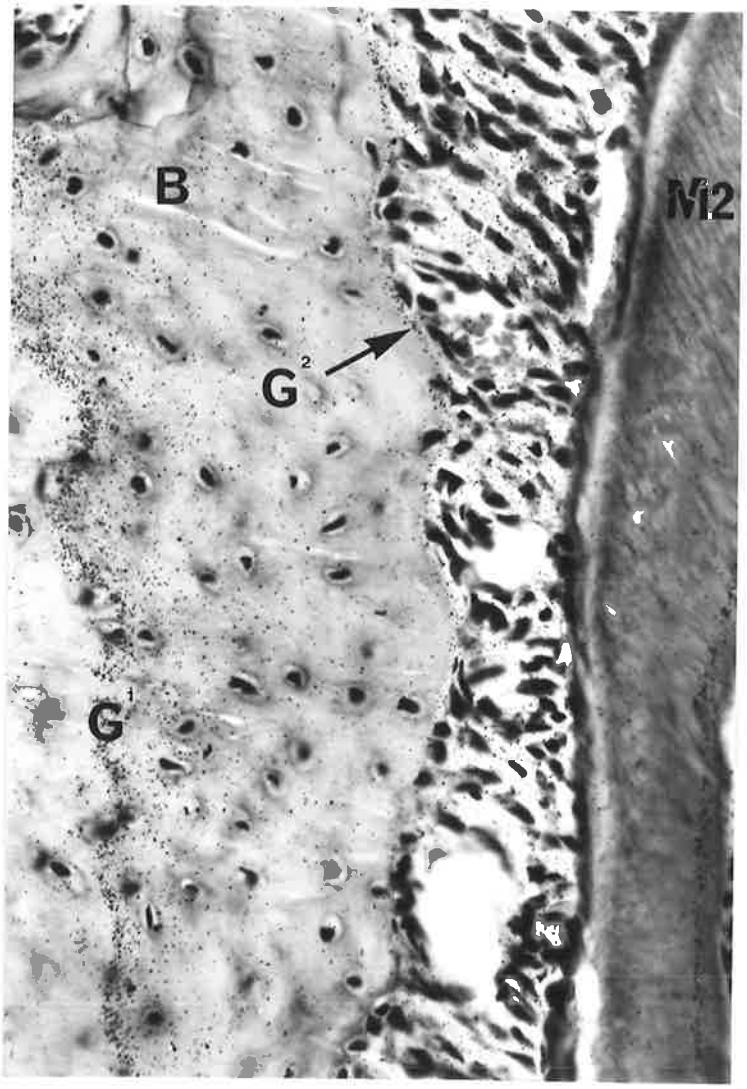


Figure 43. A mesiodistal radioautograph of a depository surface facing the second molar (M2). Two lines of silver grains G^1 and G^2 are present within bone. G^2 lies at the alveolar bone (B) - periodontal ligament interface.

H & E, x400.

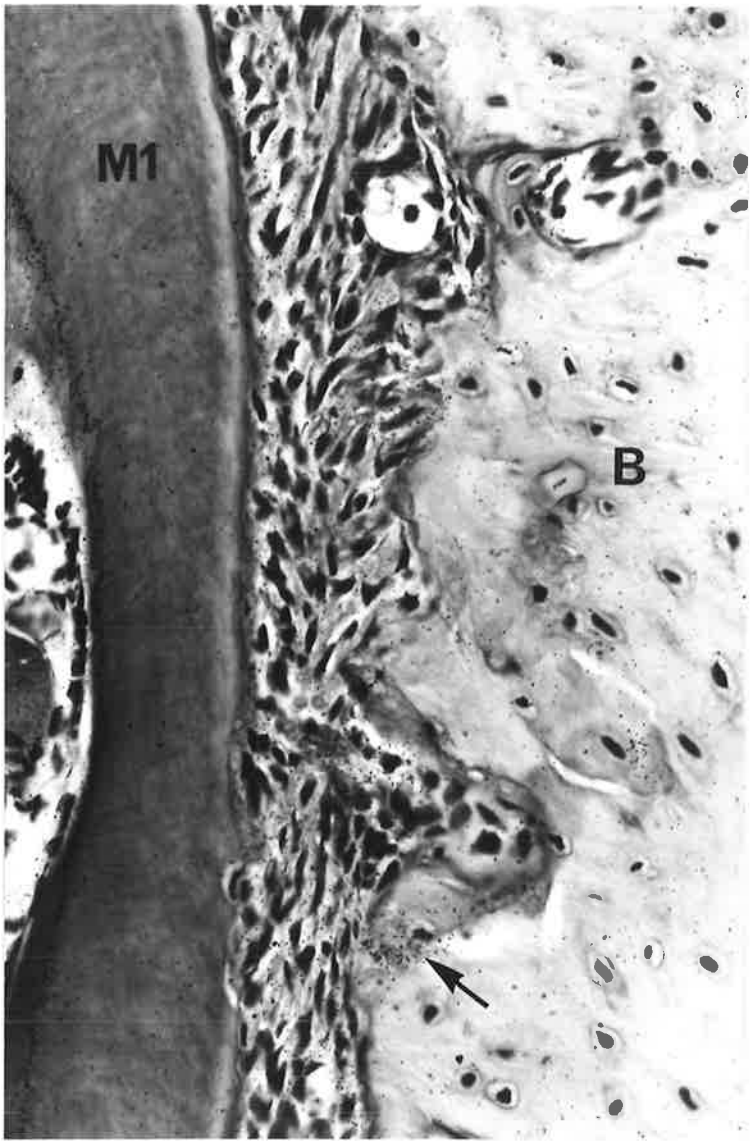


Figure 44. A mesiodistal radioautograph of a resorptive surface facing the first molar (M1). No silver grains are apparent at the bone (B) - periodontal ligament interface except for areas of spot deposition (arrowed) maintaining fibre attachment.

H & E, x400.

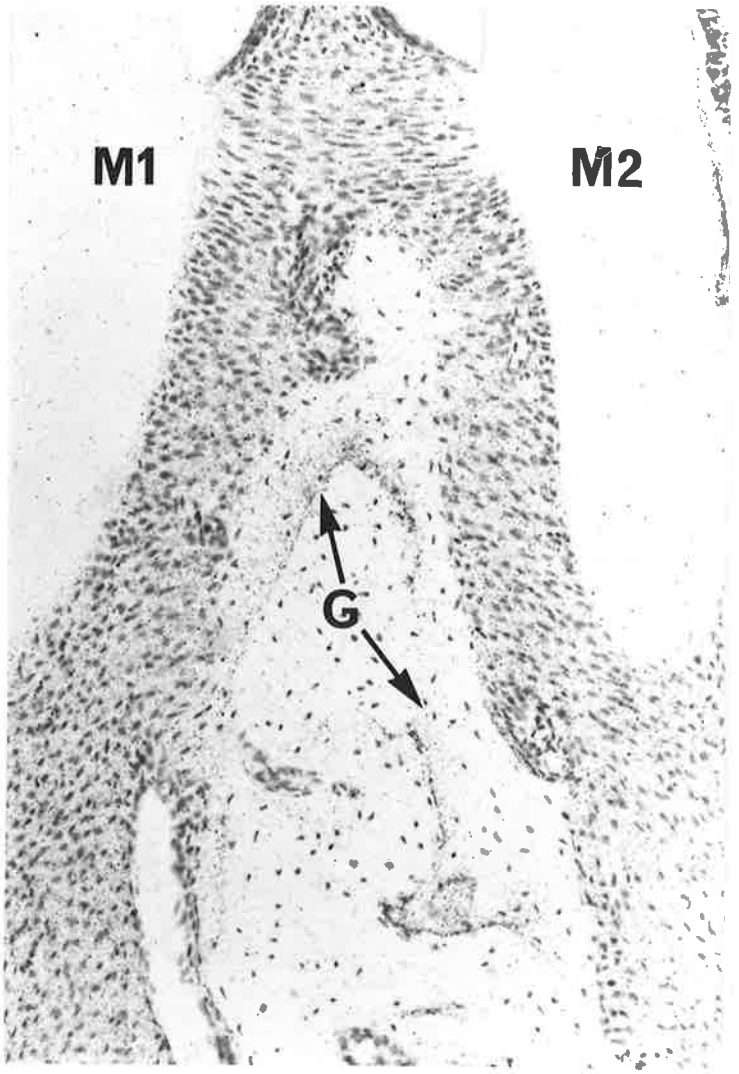


Figure 45. A mesiodistal radioautograph close to the lingual aspect of interdental septum between first and second molars (M1, M2) revealing silver grains (G) and hence bone deposition on both mesial and distal facing surfaces.

Nuclear fast red, indigo-carmine, x120.

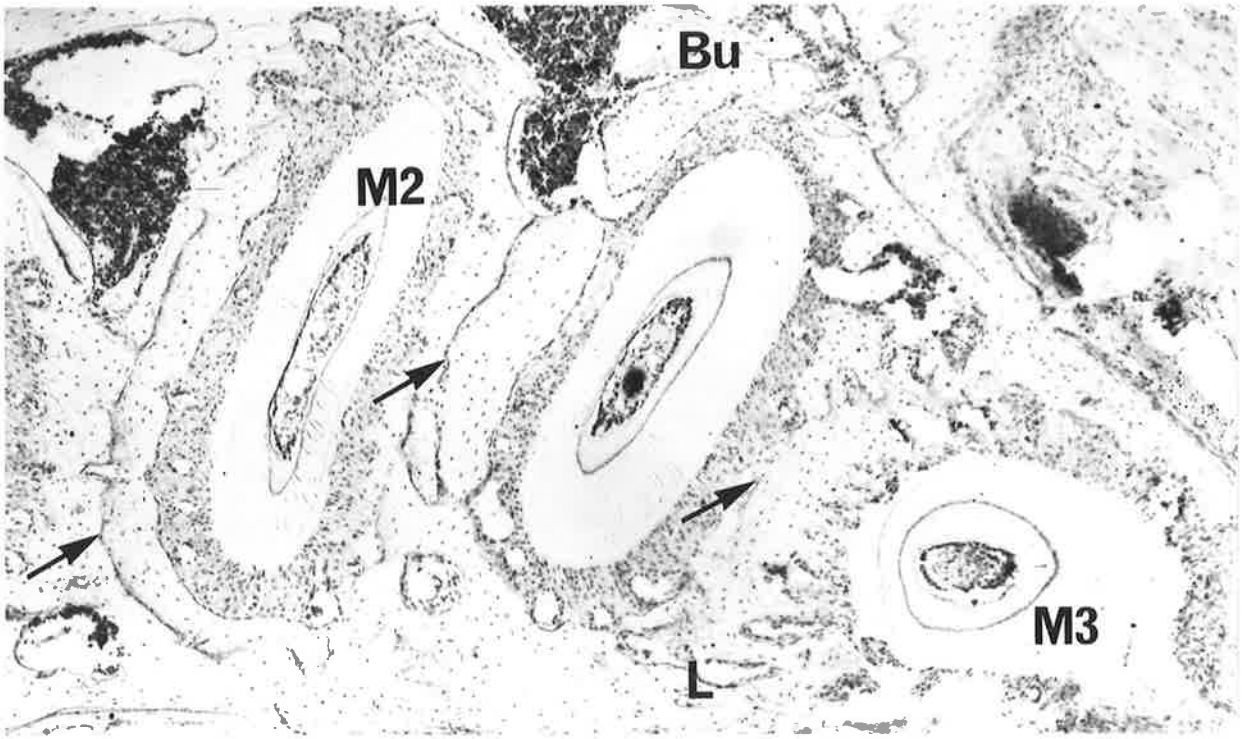


Figure 46. A horizontal radioautograph through the mid-root level of the second and third molars (M2, M3) of a 40-day control mouse revealing encircling silver grains (arrowed) indicating bone deposition at distal and buccal facing bone surfaces. Bu, buccal; L, lingual.

Nuclear fast red, indigo-carmine, x120.

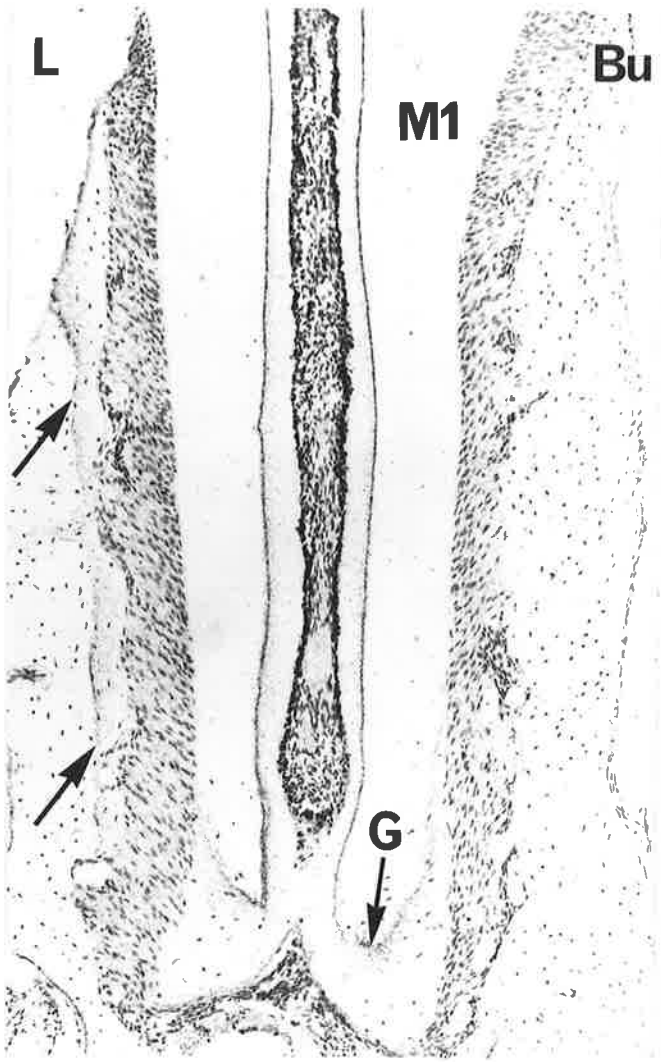


Figure 47. A buccolingual radioautograph through the distal root of the first molar (M1) showing silver grains (arrowed) lining the buccal facing surface of bone. Silver grain lines (G) within dentine are continuous into cementum. Bu, buccal; L, lingual.

Nuclear fast red, indigo-carmine, x120.

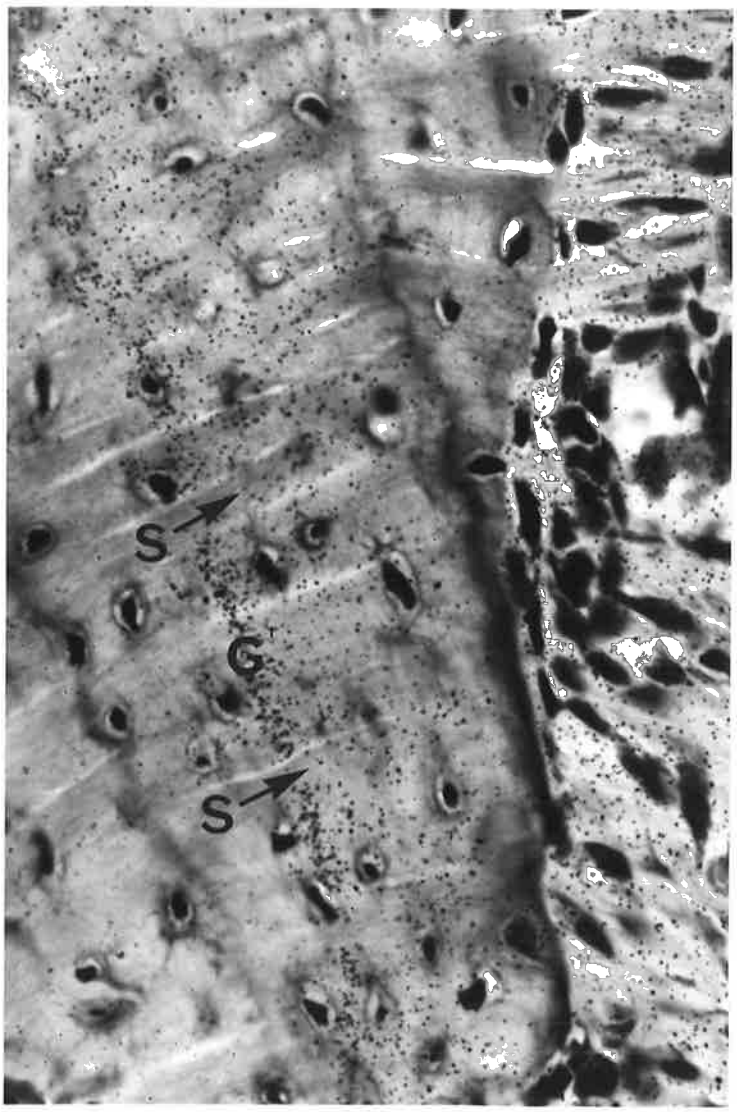


Figure 48. An area of newly deposited bone is shown in which silver grains (G^1) from the initial administration of proline are related to embedded Sharpey fibres (S).

H & E, x400.

LATHYRITIC ANIMALS.

Periodontal Ligament:

The density of silver grains present over the lathyritic periodontal ligament appeared similar to the density over the periodontal ligament of control mice (Fig. 49). The concentration of grains was indicative of continuing proline turnover.

Silver grains were also found in association with the accumulations of lathyritic material and suggested a possible collagen content. Small collections of the material were uniformly covered with label. However, in larger masses, silver grains were concentrated towards the periphery adjacent to the pallisading cells and produced alternating areas of light and heavy grain density (Fig. 4).

Dental Pulp, Dentine and Cementum:

The dental pulp retained a moderately high coating of silver grains (Fig. 49). Sites of dentine formation were demarcated by lines of silver grains. The amount of dentine deposited increased throughout the experimental period and reflected continuing tooth formation.

Similar to control mice, the initial line of label continued into cellular cementum following the contour of the root apex to its lateral margin (Fig. 60). A

second line of grains was observed in the pre-dentine layer (Fig. 49) and at the apical root tip (Fig. 58). Acellular cementum surrounding the majority of the root was devoid of silver grains.

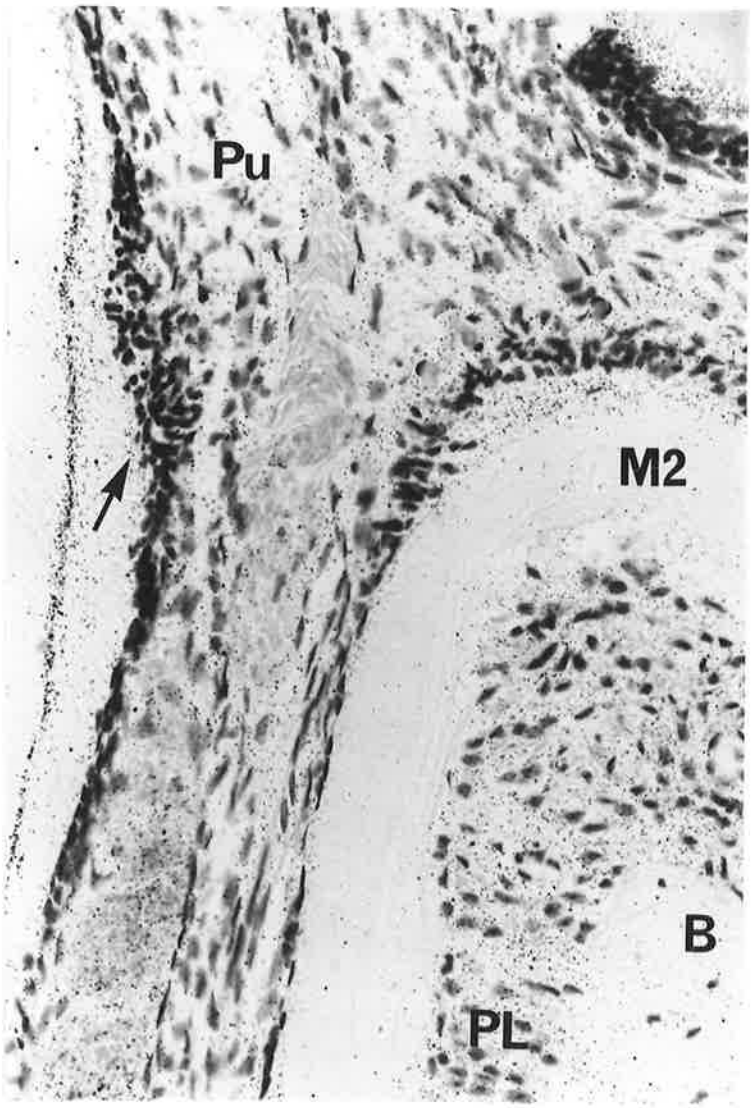


Figure 49. Silver grain distribution in the periodontal ligament (PL) and pulp (Pu) of the second molar (M2) of a 40-day lathyritic animal. Dentine formation (arrowed) is continuing. (Compare with Fig. 39).

Nuclear fast red, indigo-carmine, x350.

Alveolar Bone:

The presence of radioautographic label was seen over alveolar bone. However, the distribution was altered considerably (Fig. 50) in comparison with control mice by a change in alveolar bone surface activity. In mesiodistal sections the initial dose of tritiated proline was distributed in a similar manner to the first administration in control animals (Fig. 51). During the lathyritic period distal facing bone surfaces that originally incorporated radioactive proline appeared to change from depository to resorptive in nature which resulted in a loss of bone label (Figs. 52, 53).

In mice lathyritic for forty days silver grain loss reduced the label to areas of the alveolar crest and bone surrounding the root apices. The gradual change in the distribution of the label could be seen throughout the experimental period (Figs. 51, 52, 53, 54) until only a small line crossed the crests of the interdental and interradicular septa (Fig. 54).

The second administration of tritiated proline revealed no silver grain deposition at mesial facing bone surfaces (Fig. 55). However, some bone activity was continuing at the distal facing surfaces as determined by isolated accumulations of label (Fig. 56). In the main, resorption was the chief characteristic of this surface. It was evident that a deposit of silver grains was present

at the alveolar crests (Fig. 57) and the base of the sockets (Fig. 58) in response to continuing bone deposition at these sites.

Horizontal sections also revealed the change in radioautograph label distribution. However, the appearance of the initial silver grain line depended on the level of section. Remnants of the original marker were present in the crestal half of alveolar bone (Fig. 59). The apical areas in severely lathyritic mice contained no evidence of silver grains. The second administration of label disclosed the inactivity of proline at mesial and distal facing surfaces.

In buccolingual sections silver grains were seen associated with the alveolar crest bone and bone at the root apex (Fig. 60). There was no indication of proline turnover in either the buccal or lingual facing surfaces of the alveolus. It was noted that bone deposition was continuing on the buccal and lower borders of the mandible.

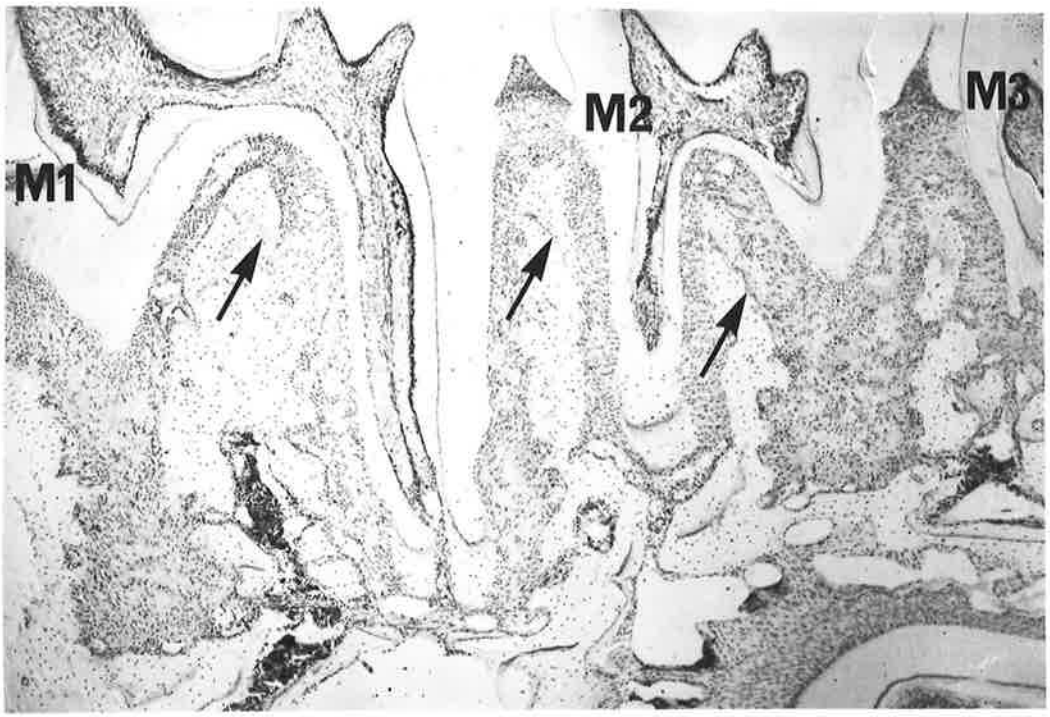


Figure 50. A mesiodistal radioautograph of the three molars (M1, M2, M3) of a 40-day lathyritic mouse revealing a change in the label distribution (arrowed). Due to increased resorptive activity of alveolar bone silver grain deposition ceased at the distal facing bone surfaces. The resorptive activity eventually removed the initial silver grain line leaving the distribution pictured. (Compare with Fig. 40).

Nuclear fast red, indigo-carmine, x60.

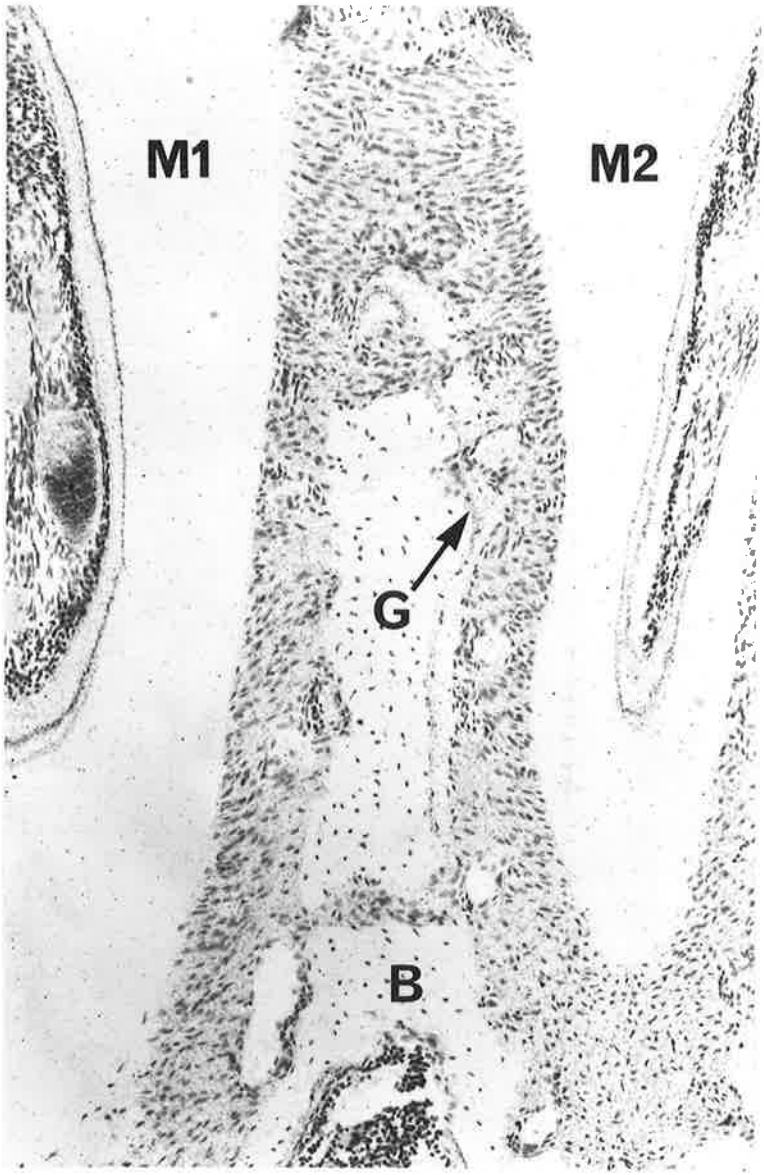


Figure 51. A mesiodistal radioautograph of the interdental septum (B) between the first and second molars (M1, M2) of a 10-day lathyritic mouse in which silver grain distribution (G) is similar to control animals. (Compare with Figure 41). Silver grains line the alveolar crest and distal facing bone surface to the apical region of M2.

Nuclear fast red, indigo-carmine, x120.

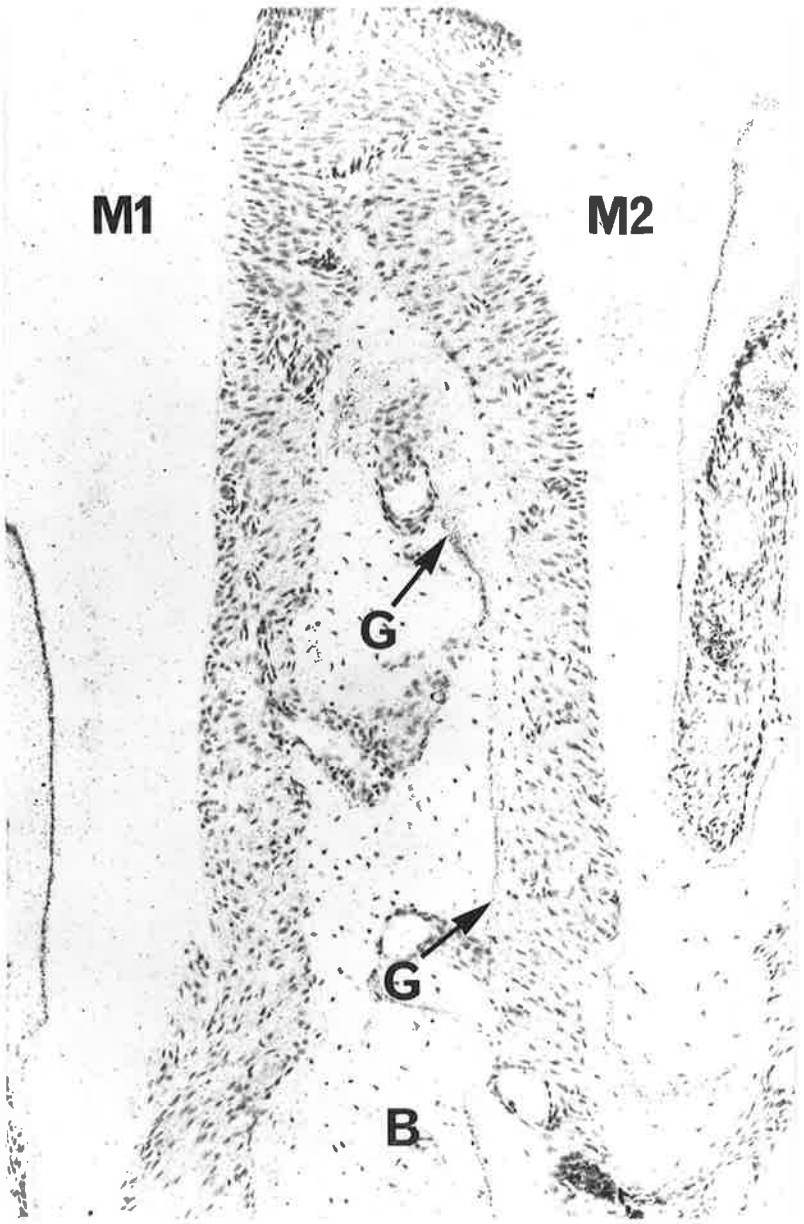


Figure 52. A mesiodistal radioautograph of the interdental septum (B) between the first and second molars (M1, M2) of a 20-day lathyritic mouse in which bone deposition indicated by the silver grain line (G) is continuing at the alveolar crest but less so lateral to the root apex of M2.

Nuclear fast red, indigo-carmine, x120.

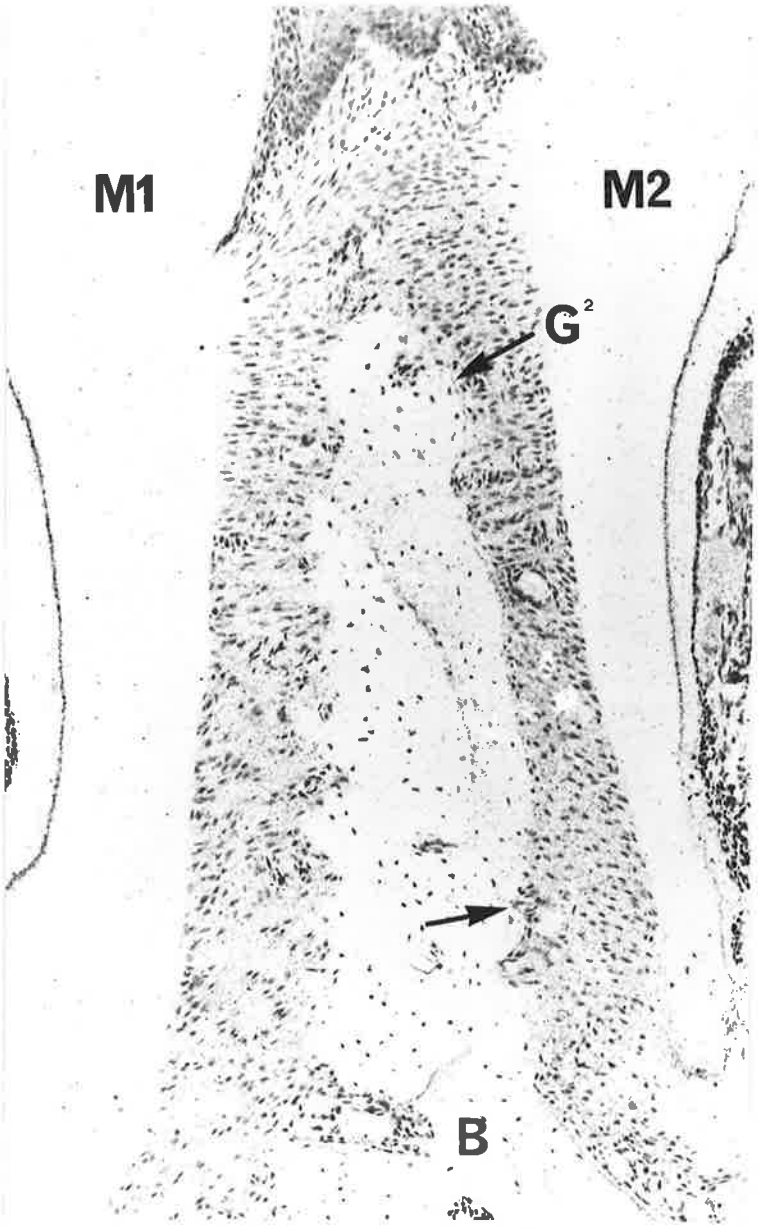


Figure 53. A mesiodistal radioautograph of the interdental septum (B) between the first and second molars (M1, M2) of a 30-day lathyritic mouse revealing a loss of the initial silver grain line (arrowed) lateral to the root apex of M2. Bone deposition (G^2) is continuing at the alveolar crest.

Nuclear fast red, indigo-carmine, x120.

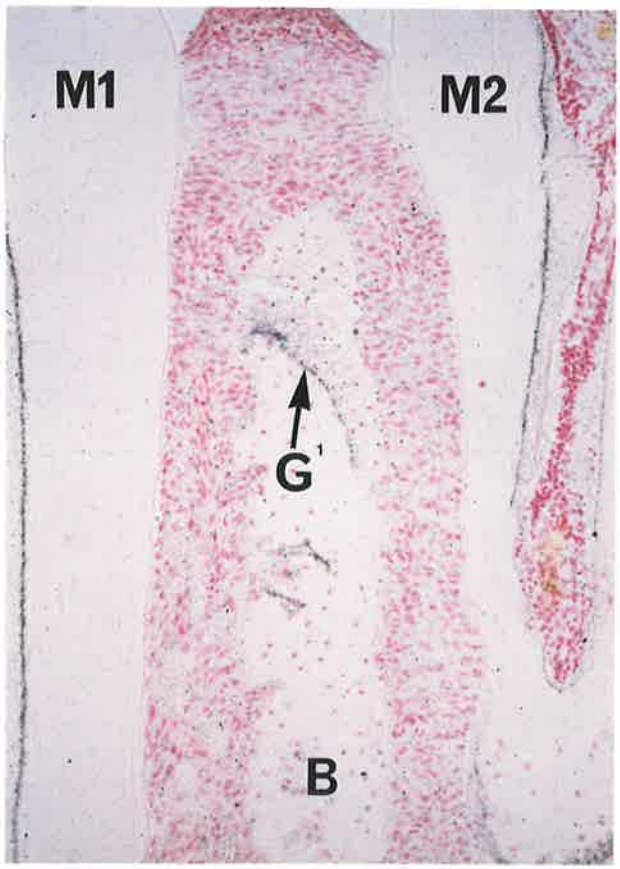


Figure 54. A mesiodistal radioautograph of the interdental septum (B) between the first and second molars (M1, M2) of a 40-day lathyritic mouse in which resorption of the distal facing bone surface has left a small segment of the initial silver grain line (G^1) diagonally crossing the alveolar crest. (Compare with Fig. 42).

Nuclear fast red, indigo-carmine, x120.

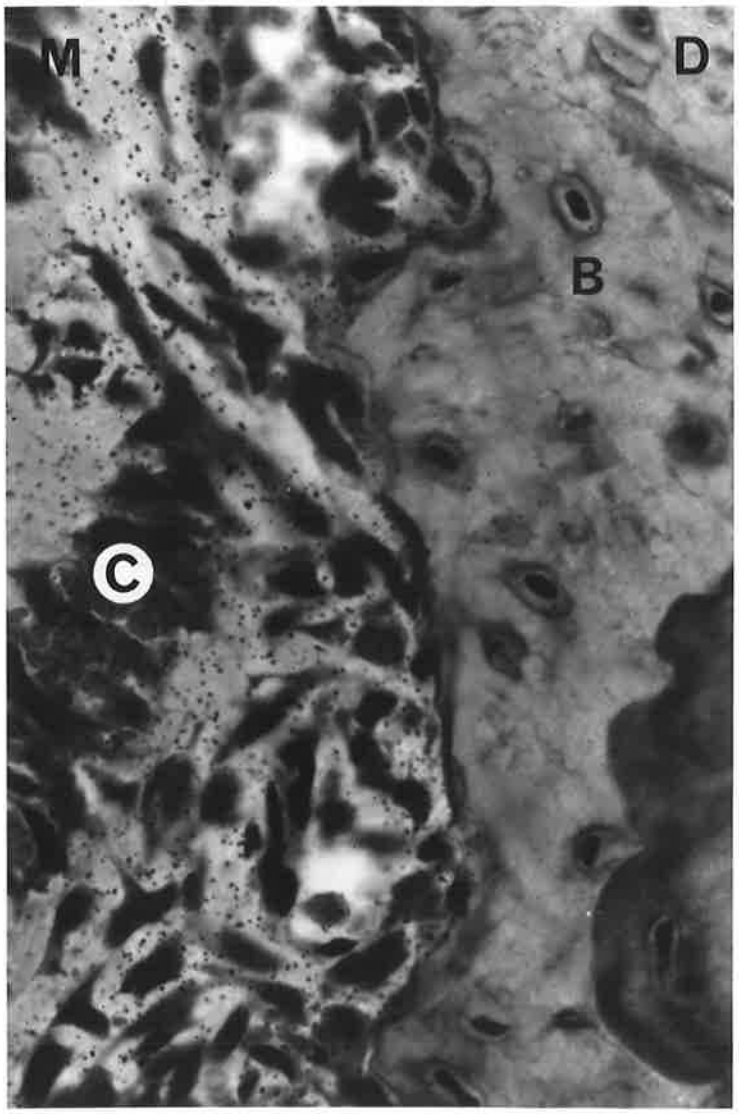


Figure 55. A radioautograph of mesial facing bone (B) of a 40-day lathyritic mouse revealing no silver grain deposition at the bone surface. The resorptive nature of this surface was maintained during lathyrism.
M, mesial; D, distal; C, pallisading cells.

H & E, x450.

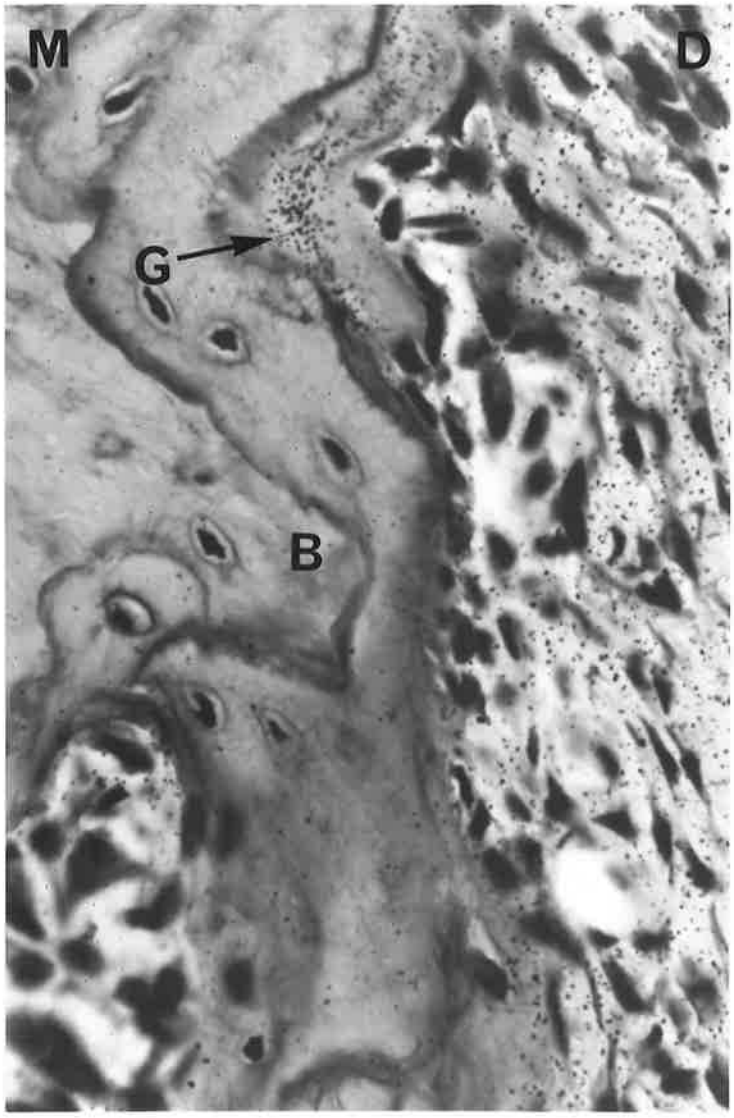


Figure 56. A radioautograph of distal facing bone (B) of a 40-day lathyritic animal which contained no silver grains except in isolated pockets (G) of spot deposition. The majority of this surface changed from depository to resorptive in nature. M, mesial; D, distal.

H & E, x400.

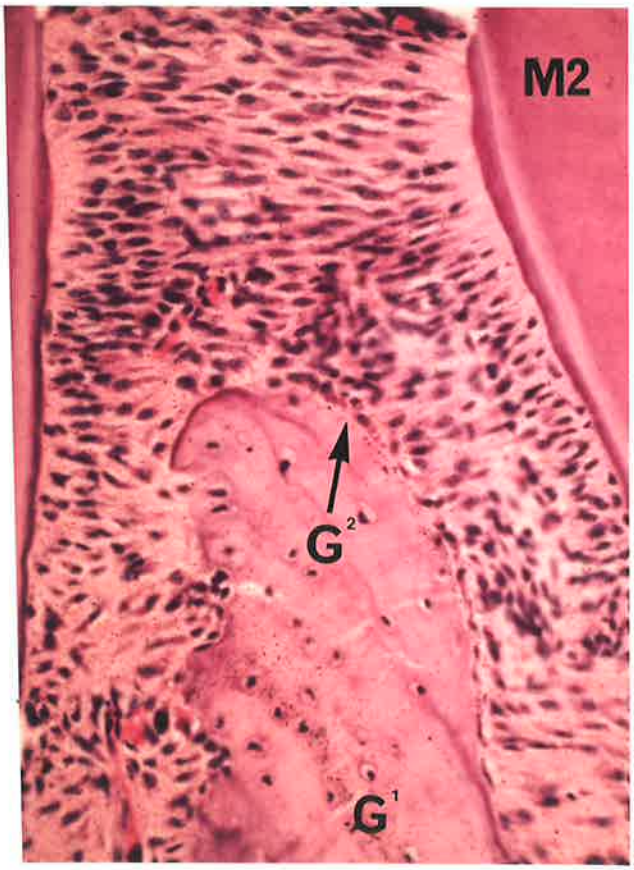


Figure 57. A radioautograph of the alveolar crest between the first molar and second molar (M2) of a 40-day lathyritic mouse showing continuing bone deposition at the crest as revealed by the most recently deposited line of silver grains (G^2). G^1, initial line of silver grains.

H & E, x400.

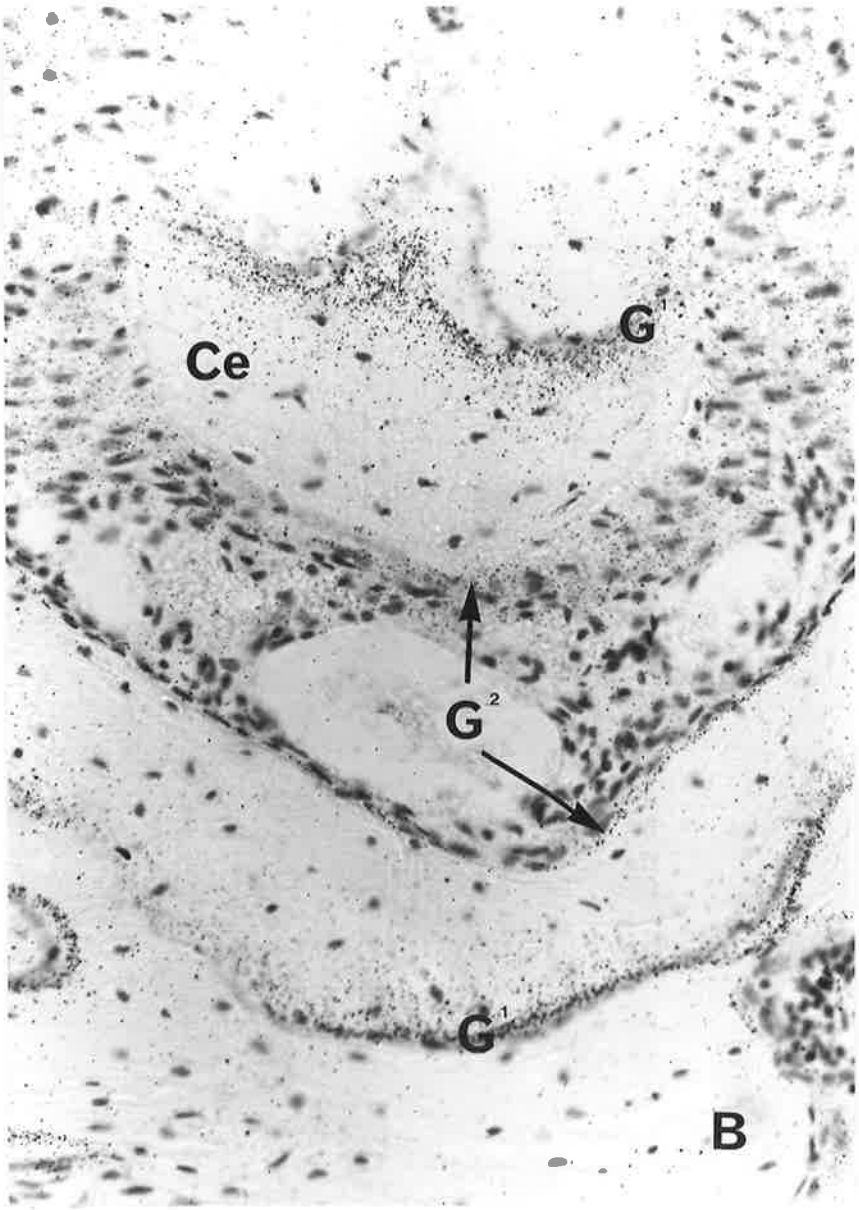


Figure 58. A radioautograph of the apical area of the second molar of a 40-day lathyritic mouse showing the initial line of silver grains (G^1) within cementum (Ce) and alveolar bone (B). Continuing cementum and bone deposition is reflected by the most recently deposited line of label (G^2).

Nuclear fast red, indigo-carmine, x400.

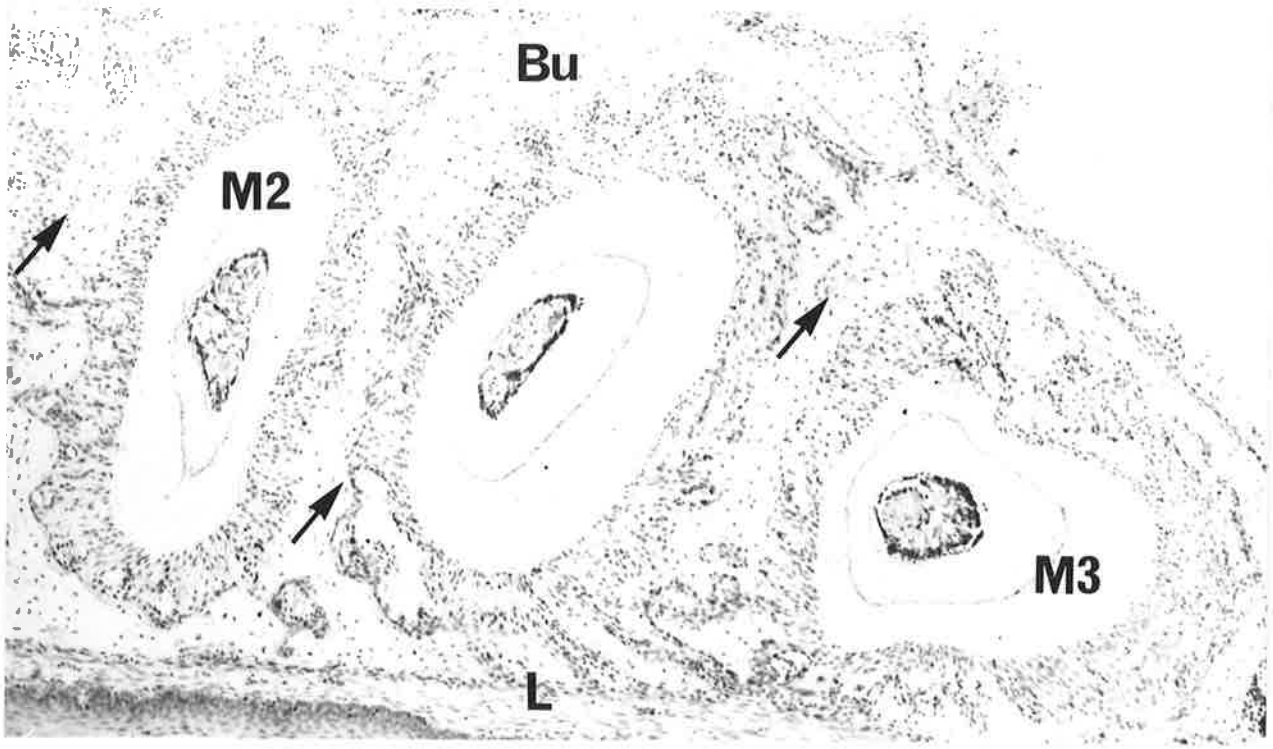


Figure 59. A horizontal radioautograph at about the level of the mid-root of the second and third molars (M2, M3) of a 40-day lathyritic mouse showing the remnants of the initial silver grain lines (arrowed). (Compare with Fig. 46).
Bu, buccal; L, lingual.

Nuclear fast red, indigo-carmine, x120.

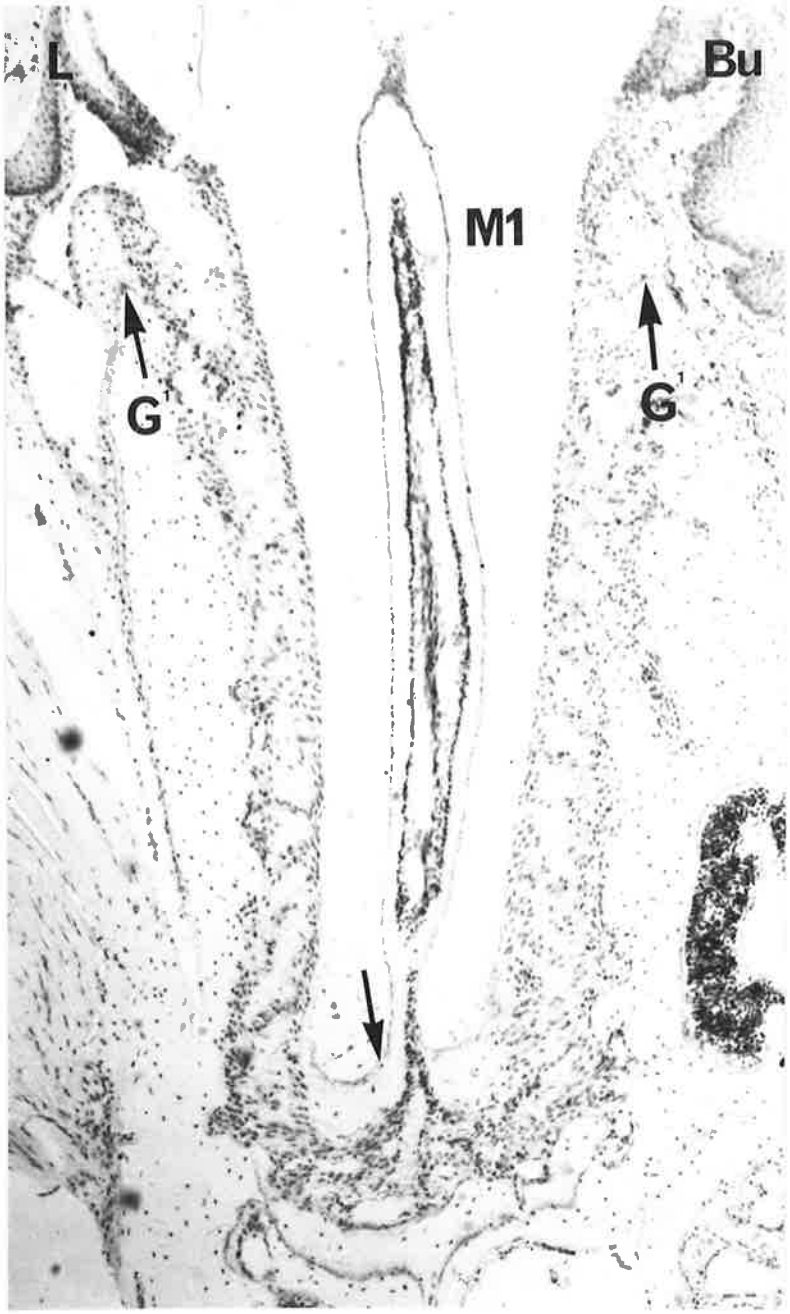


Figure 60. A buccolingual radioautograph of the distal root of the first molar (M1) of a 40-day lathyritic mouse showing no silver grain activity at the buccal and lingual facing bone surfaces. Remnants of the initial grain line (G^1) are present at the alveolar crests. The continuity of the initial grain line is still evident through dentine into cementum (arrowed). (Compare with Fig. 47). Bu, buccal; L, lingual.

Nuclear fast red, indigo-carmine, x120.

CHAPTER 6

DISCUSSION.

RADIOAUTOGRAPHY.

The localization of marking agents at depository bone sites is an essential criterion for determining bone formation (Cleall, Perkins and Gilda, 1974). It has been found that amino acids are rapidly processed intracellularly and secreted into the extracellular spaces (Stallard, 1963; Carneiro and Fava de Moraes, 1965). Since bone matrix comprises 95% of protein collagen (Hancox, 1972) areas of radioactive amino acid incorporation have been interpreted as representing sites of active collagen synthesis and bone formation (Stallard, 1963; Carneiro and Fava de Moraes, 1965; Tonna, 1974; 1975; 1976). Once incorporated into bone matrix radioautographic label remains stable until removed by remodelling resorption (Tonna, 1974). Valuable information on alveolar bone activity was therefore available using a topographical labelling technique.

Radioautographic labelling is the placement of a photographic (nuclear) emulsion containing silver halide crystals in close contact with a source of radiation (Krutchkoff, 1972; Rogers, 1979). The emitted energy of radioactive decay interacts with the emulsion to

produce silver ions. These ions are subsequently precipitated as metallic silver on exposure to a reducing agent (developer).

The radioautography of the present study utilized tritiated proline as the radioactive isotope and bone marker. Tritium, an isotope of hydrogen with a half-life of 12.3 years, has been commonly used in investigations requiring radiotracers to determine sites of protein activity (Rogers, 1979). Krutchkoff (1972) stated that the bonding capacity of the tritium nucleus was relatively poor and led to nuclear instability. Stability was achieved by decay in a one-step process emitting a β-particle of a low energy of 18 keV (Rogers, 1979). Because of its low energy the β-particle had a short range and Krutchkoff (1972) determined that the practical limit of β-decay was approximately 2.5 micrometres. Furthermore, a moderately sensitive Ilford K2 nuclear emulsion of a thickness greater than 3 micrometres produced silver grains very close to the point of decay and hence provided high resolving power.

According to Boren, Wright and Harris (1975) and Rogers (1979) variables may be inadvertently introduced into the radioautographic technique. In relation to tissue preparation the quality of radioautographs is affected by vascular absorption from the intraperitoneal injection, fixation, decalcification and specimen thickness. Since in the present study tissue preparation was

standardized, any untoward effect would have been consistent throughout all specimens and enabled valid comparisons to still be made. The processing of radioautographs may be affected by the exposure time, temperature and relative humidity, development, fixation and staining procedures. High concentrations of silver grains were revealed over the periodontal ligament with minimal background radiation using an exposure period of thirty-five days. The observance of controlled conditions and technique produced quality radioautographs with no apparent latent image fading.

The turnover of radioactive amino acids within the periodontium was shown to be rapid (Crumley, 1964; Carneiro and Leblond, 1966; Skougaard, Levy and Simpson, 1970; Skougaard and Levy, 1971; Kameyama, 1975; Sodek, 1976). Since amino acids were also incorporated into non-collagenous proteins (Eastoe, 1976; Orlowski, 1976), they had often questionably been used as an absolute measure of collagen turnover within the periodontal ligament. Carneiro and Leblond (1966), Rippin (1976; 1978) and Sodek (1976) produced reliable data concerning collagen turnover within the periodontium using a highly specific collagenase. As no highly specific techniques were undertaken and radioautographically collagen turnover had been widely reported, no quantitative assessment was performed using material of the present study. Furthermore, the lack of sufficient numbers of experimental animals cast doubt over any statistical analysis of collagen turnover.

HISTOLOGY OF THE NORMAL PERIODONTIUM.

Principal Fibres:

In the present study size variation in the principal fibre bundles of the periodontal ligaments was seen on either side of the interradicular and interdental bone. Principal fibre bundles adjacent to distal facing bone surfaces were large in diameter and continuous with penetrating Sharpey fibres (Fig. 13), whereas principal fibres adjacent to mesial facing bone were small in diameter and appeared to attach to the bone surface (Fig. 12). These findings had been previously reported by Dunstan (1975) in relation to mice yet Edwards (1977) reported the reverse findings in relation to human teeth. Edwards (1977) related large principal fibres to the mesial facing bone surface. Dunstan (1975) attributed his findings of principal fibre variation to distal tooth migration in response to a distal force vector. Similarly, a mesial force vector in humans may account for the mesial tooth migration (Orban, 1976) and the large diameter of principal fibres adjacent to mesial facing bone surfaces (Edwards, 1977).

Shackleford (1971) reported an "indifferent" fibre plexus comprised of many fine collagen fibrils within the rat periodontal ligament. Once under stress these fine fibrils aggregated into larger fibres of definite orientation. Although the appearance of these "indifferent" fibres may have been a result of Shackleford's (1971)

method of tissue preparation, Grant and Bernick (1972) also reported varying morphology of the developing rat periodontal ligament. Prior to occlusal contact all periodontal ligament fibres were of similar size and arrangement. However, with the advent of occlusal load differences in fibre size and orientation became apparent at mesial and distal bone surfaces. These results suggested that tooth function was important in the structure of the periodontal ligament. If this hypothesis is taken further, functional variations may account for the morphology of periodontal ligaments on either side of the alveolar septa and provide the basis of Dunstan's (1975) concept of a distal force vector in operation. According to the time-honoured "pressure-tension" theory (Baumrind, 1969), sites of periodontal tension caused stretching and aggregation of principal fibres. Since in the present study large principal fibres were observed in the periodontal ligament adjacent to the distal facing bone surface, this ligament may be under tension. Sites of periodontal compression resulted in fibre disorientation which in the present study was seen in the periodontal ligament adjacent to the mesial facing bone surface.

Although the "pressure-tension" theory is not universally accepted (Baumrind, 1969), the possibility of functional variations within periodontal ligaments adjacent to mesial and distal facing bone surfaces as an explanation of the morphologic pattern cannot be ignored.

Intermediate Plexus:

In order to account for tooth eruption and migration the existence of an intermediate plexus was postulated by Sicher (1923). The present study found no evidence of an anastomotic meeting of principal fibres in the centre of the ligament. Principal fibres were seen to branch and intermingle with their neighbours yet often could be traced across the entire width of the periodontal space (Fig. 11). However, histology presents a static visual interpretation which may not be a true representation of dynamic fibre turnover within the periodontal ligament.

Transalveolar Fibres:

Cohn (1972a) and Dunstan (1975) reported the existence of a tooth-to-tooth fibre system that pierced the interdental and interradicular septa. Recently, the developmental nature of these transalveolar fibres was described by Johnson (1980) and the concept of dental attachment to bone was revised. Evidence for the existence of transalveolar tooth-to-tooth fibres could not be found in the present study. Sharpey fibres which penetrated bone at a depository surface crossed the alveolar septa but appeared to terminate at either a reversal line within bone (Fig. 23) or at a resorptive surface rather than enter the opposite periodontal ligament (Fig. 24). It is possible that Sharpey fibres deviated in their course and gave the impression of

termination. However, in horizontal sections, penetrating fibres were not seen to deviate but followed a direct line within bone (Fig. 25).

Johnson (1980) in his developmental study claimed transalveolar fibre formation to be essentially complete in thirty-day old mice. Within the crest of the interdental septa he described an "upright V" pattern of transalveolar fibres caused by a change in direction of alveolar crest dentoalveolar fibres within bone. A similar V-pattern of perforating fibres was seen in the present study (Fig. 26). However, this pattern was not consistent throughout serial sections of any one specimen. In mesiodistal sections approximating the lingual aspect of the teeth Sharpey fibres penetrated both mesial and distal facing bone surfaces. Accordingly, these surfaces were classified as depository in nature as a result of a buccolingual vector of migration. The penetrating Sharpey fibres were seen to merge but not unite within bone (Fig. 26). Sections examined further buccally revealed less penetration of fibres entering from the mesial facing bone surface until only surface attachment of periodontal fibres occurred. This decreasing penetration of fibres into mesial facing bone surfaces and the increasing depth of fibre penetration into distal facing bone surfaces was clearly seen in horizontal sections (Fig. 25). Neither Cohn (1972a) nor Johnson (1980) studied the horizontal morphology of the transalveolar fibres and therefore their findings are based on an

incomplete examination of their material. The present study concurs with the findings of Bernick, Levy, Dreizen and Grant (1977) who suggested that a tooth-to-tooth transalveolar fibre system did not exist and that fibres penetrating bone were related to the formative and later to the functional state of the periodontium.

HISTOLOGY OF LATHYRISM.

The morphologic changes of the lathyritic periodontal ligament have been widely reported in the literature (Krikos et al, 1958; Gardner et al, 1958; Barrington and Meyer, 1966; Heller and Nanda, 1979). After examining ground substance (Tanzer, 1965), amyloid, fibrinoid and hyalin (Krikos, 1964), current opinion held that the lathyritic material comprised non-fibrous collagen. The amorphous masses surrounded by pallisades of cells were also seen in the present study (Fig. 4). Polarizing microscopy revealed birefringent material within the lathyritic accumulations (Fig. 20) and so disagreed with the non-fibrous concept previously reported by Gardner (1960). However, rather than arising from new collagen synthesis these birefringent fibres may have been remnants of the normal periodontal ligament fibres.

A new concept by Kardos and Simpson (1980) suggested that the collagen of the periodontal ligament was present in a labile or partially polymerized form and

so exhibited the rheologic properties of a thixotrophic gel. Although this theory remains unproven it is possible that the physicochemical lathyritic alteration of collagen may have some rheologic effect. The collections of lathyritic material may therefore comprise an altered phase of collagen.

In agreement with the observation of Krikos et al (1958) the staining of the lathyritic material was similar to that of collagen. An additional finding of the present study was the high number of silver grains over this material particularly adjacent to the cells (Fig. 4). Although tritiated proline labels other proteins besides collagen these observations provide supportive evidence that collagen is a component of these amorphous masses. Although the difficulty of tissue sampling within the periodontal ligament is well recognized (Sodek, 1976) a biochemical assay would provide absolute proof as to the nature of this material.

According to Herovici (1963) his technique permitted the staining of immature and mature collagen, although he could not entirely account for the differential effect. Craik and McNeil (1966) determined that tensional effects played a role in the staining reaction. Experiments in which young rat tail collagen was stretched produced the red staining characteristics of acid fuchsin. Given the biochemical specificity of histologic stains, in neither control nor lathyritic sections was the methyl

blue colour of "young" collagen seen. The red staining of collagen by acid fuchsin predominated. This was considered unusual provided that "young" collagen can be equated with the increased content of immature collagen within the periodontal ligament produced by the lathyritic inhibition of the collagen maturation process (Piez, 1968; Levene, 1973). Tissue fixation using Bouin's solution enhances polychrome staining (Luna, 1968). However, the presence of picric acid may have caused the decreased effectiveness of Herovici's technique. Herovici (1963) reported an improved staining reaction following the use of 5% sodium thiosulphate prior to staining, when Bouin's solution had been used as a fixative. The present finding suggests that the immersion of specimens in 70% alcohol for twenty-four hours after fixation may have been insufficient to remove excess picric acid (Beertsen and Tonino, 1975). Lillie (1965) recommended immersion for two to three days.

Lathyritic Distribution:

Disagreement exists as to the distribution of the lathyritic lesion, even though morphologic descriptions of the lathyritic periodontal ligament have been extensive. Krikos et al (1958) reported that lathyritic changes were present within three days and that they were limited to the apical one third of the ligament. The effects increased in severity up to a maximum at thirteen days when most of the ligament was found to be

greatly disorganized. Krikos et al (1958) did not refer specifically to the supracrestal areas. Gardner et al (1958) described the appearance of lathyritic material after one week of lathyrism and the arrangement of cells into pallisades after three weeks.

The present study examined lathyrism up to forty days. In agreement with Krikos et al (1958) the initial lesion with cell orientation was found in the periapical region (Fig. 5). Thereafter the lathyritic lesion gradually and progressively spread up the periodontal ligament but failed to involve the supracrestal area (Fig. 6). In both the time period involved and the severity of the change, the results of the present study differ from those of Krikos et al (1958) and Gardner et al (1958). The differing results may be explained on the basis of age, sex and species of the experimental animals (McCallum, 1965) and on the lathyrogen used.

Lathyrism does not affect collagen synthesis but rather disturbs the aggregation of newly formed collagen (Levene, 1973). Accordingly, it was considered that tissues with a higher turnover of collagen were more susceptible to the effects of lathyrism. The periodontal ligament has a high turnover of collagen (Skougaard and Levy, 1971; Kameyama, 1975). Furthermore, there is evidence that a rate differential exists along the length of the ligament. Collagen turnover is higher

in the periapical region compared with the crestal area (Rippin, 1976). This variation in the rate of collagen turnover within the periodontal ligament provides one possible explanation for the observed progression of the lathyritic disruption.

Across the width of the periodontal space higher collagen turnover has been reported adjacent to alveolar bone (Stallard, 1963; Stahl and Tonna, 1977). Other reports differ, finding an even distribution of silver grains (Skougaard and Levy, 1971; Rippin, 1976). The lathyritic accumulations observed in the present study gathered centrally within the periodontal ligament and gradually enlarged to approach the cemental and alveolar surfaces. Although silver grain distribution appeared even across the periodontal space and occlusal effects need to be considered, the lathyritic change might suggest less mature or a higher turnover of collagen in the centre of the ligament which is perhaps supportive of the intermediate plexus concept.

The orientation of the lathyritic lesion (amorphous material and cells) within the periodontal ligament provided another curious observation. Although the rows of cells were aligned occlusoapically from cementum to bone, the long axes of the cells paralleled the usual orientation of the periodontal ligament fibres (Fig. 4). Krikos et al (1965) found that occlusal force was necessary for the morphologic appearance of the

lathyritic lesion. It is therefore possible that occlusal loading, particularly in the form of tensional effects to the fibres, may play a role in the cellular alignment. However, this explanation does not account for the apparent lack of lathyritic disruption in the incisal periodontal ligament which however is continually erupting.

Bone Changes During Lathyrism:

Reddi and Sullivan (1979) investigated the influence of β-aminopropionitrile on "matrix induced" endochondral ossification. It was found that cartilage and bone mineralization were profoundly inhibited both in rate and extent even though bone matrix formation was unaffected. It was suggested that the disturbance in bone mineralization was responsible for the bone changes during lathyrism. This conclusion was supported by Goren et al (1977) in a developmental study of lathyritic chicks.

The application of this finding suggests that there is active lathyritic resorption of alveolar bone. The tissue incorporation of tritiated proline provides evidence of bone matrix production. However, in the present study the injection of label twenty-four hours prior to animal sacrifice produced no silver grains in either mesial or distal facing bone surfaces adjacent to severe lathyritic periodontal ligament disturbance (Figs. 55, 56). If the sole effect of lathyrism is the

blockage of the bone mineralization process silver grains could be expected to be deposited in areas of normal bone matrix activity and in particular the distal facing surface. The lack of silver grains in mesial and distal surfaces suggests that bone matrix production has ceased and that active resorption was occurring. Dentine and cementum matrix formation were seen to continue as evidenced by radioautographic label accumulation in pre-dentine and pre-cementum (Figs. 49, 60). This was possibly a reflection of a different type or structural arrangement of collagen in dental tissues (Carmichael and Dodd, 1974) or merely a continuation of dental matrix production. The cessation of bone matrix formation suggests that other factors are involved in alveolar bone changes besides a defect in mineralization.

Cohn (1965) studied disuse atrophy of the periodontium in mice by selectively extracting molars on one side of the mouth. He reported a disorientation and detachment of the fibres of the periodontal ligament with extensive resorption of alveolar bone. More recently, Levy and Maillard (1980) described similar effects of occlusal hypofunction on the periodontium of rats. Hypofunctional tooth migration was continuing at a higher than normal rate but appeared related to the supra-eruption of the unopposed teeth.

The effects described by Cohn (1965) and Levy and Maillard (1980) bear a striking similarity to

the periodontal changes in lathyrism. Periodontal ligament disorganization and resorptive bone changes were routine findings. It is possible that a form of disuse atrophy is responsible for the morphologic changes of lathyritic bone.

The strength of lathyritic collagen is impaired (Levene and Gross, 1959; Levene, 1973). The collagenous fibres of the periodontal ligament have been assigned a possible tooth supporting role (Melcher and Bowen, 1969). Accordingly, the weakened lathyritic periodontal ligament, unable to act in its tooth supporting capacity, may not be able to resist normal occlusal forces which then become traumatic. The inability to brace the tooth against occlusal load may lead to a lack of functional stimulation or traumatic disruption to alveolar bone.

TOOTH MIGRATION IN CONTROL MICE.

Previous histologic studies have relied on Sharpey fibre patterns to provide directional information of molar tooth migration (Zwarych and Quigley, 1965; Kraw and Enlow, 1967; Quigley, 1970; Baron, 1973; Bernick et al, 1977). Sharpey fibres are formed by bone deposition around the principal fibres of the periodontal ligament which are then incorporated into the structure of bone (Garant and Cho, 1979). The possibility of considerable principal/Sharpey fibre remodelling

before or during the process of incorporation was suggested by the presence of radioautographic label lying over embedded Sharpey fibres.

The present study found Sharpey fibres penetrating the surfaces of alveolar bone facing distally and buccally as well as embedded in the alveolar crest in control animals (Figs. 22, 24, 25, 27). Accordingly, evidence of new bone formation was implied by Sharpey fibre insertions at these sites.

Areas where Sharpey fibres were tenuously attached to bone were designated resorptive in nature (Kraw and Enlow, 1967; Kurihara and Enlow, 1980). In the control mice used in the present study, tenuous Sharpey fibre relationships were found at mesial and lingual facing surfaces of alveolar bone.

The histologic pattern of Sharpey fibre relationships with alveolar bone therefore reflected bone activity in accordance with tooth migration in a distal and occlusal direction. This finding agreed with previously recorded studies in rats (Sicher and Weinmann, 1944; Zwarych and Quigley, 1965; Kraw and Enlow, 1967; Quigley, 1970) and in mice (Dunstan, 1975; Burnett, 1978).

Moreover, Sharpey fibre associations with buccal and lingual alveolar bone surfaces was indicative of bone activity and tooth movement in a buccolingual

plane. It was concluded that a hitherto infrequently recorded buccal vector of tooth migration existed.

The observation that Sharpey fibres penetrated both the mesial and distal facing surfaces of interdental and interradicular bone in sections close to the lingual aspect of the teeth suggested that these surfaces were depository in nature. This appeared inconsistent with tooth migration solely in a distal direction. However, an explanation was provided in terms of the buccal vector of drift. Since Sharpey fibres perforated the buccal facing surface of bone, it was possible to section the teeth mesiodistally such that this unusual pattern of Sharpey fibres was revealed (Fig. 61). Bone deposition was occurring on these surfaces in response to tooth migration away from the lingual cortical plate. This explanation was confirmed by sections examined farther towards the buccal aspect of the teeth in which the penetration of Sharpey fibres into mesial facing bone was seen to recede (Fig. 61) and by sections examined in a horizontal plane.

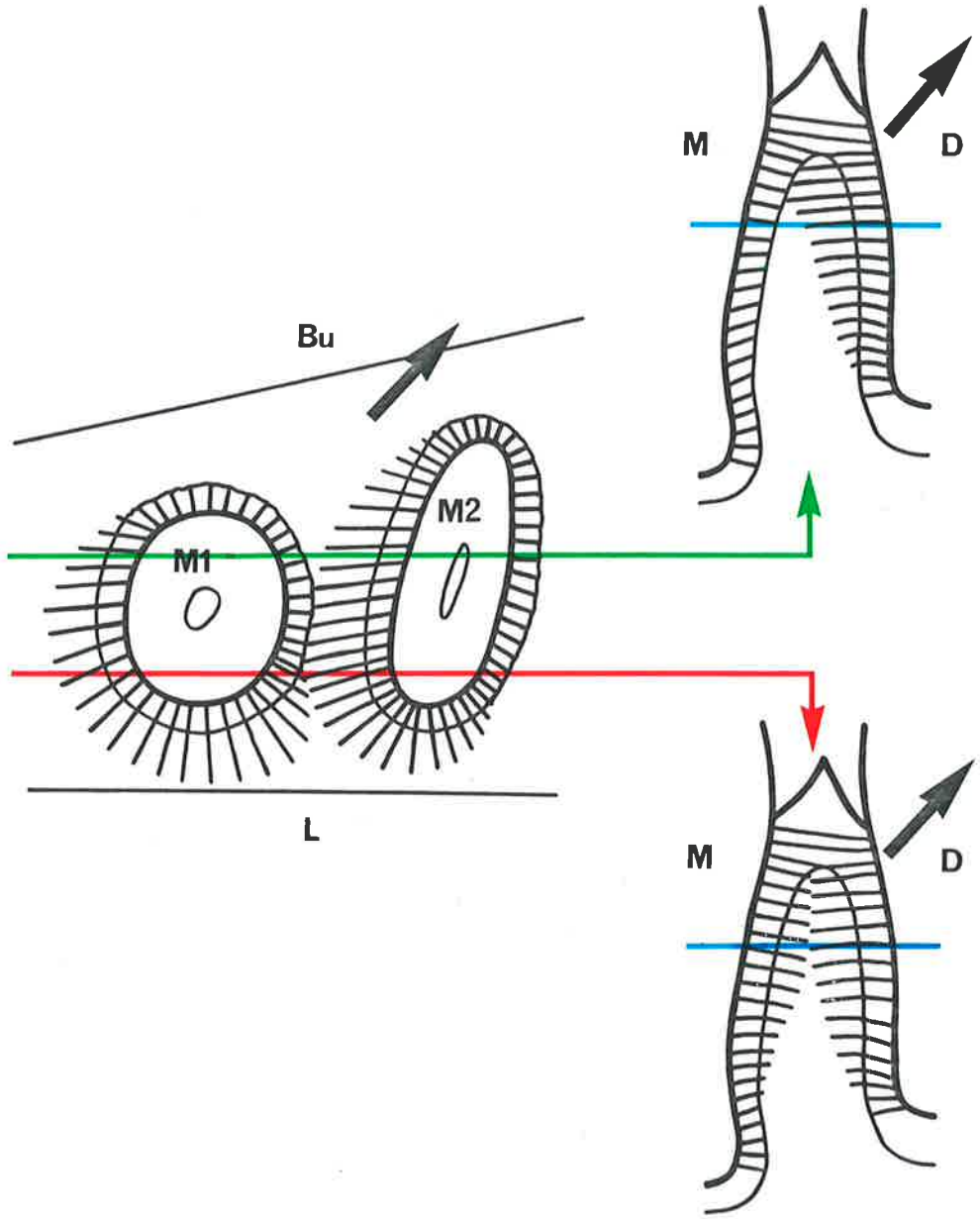


Figure 61. These schematic diagrams indicate the effect of the mesiodistal plane of section on the relationship of Sharpey fibres with alveolar bone surfaces. Sections cut from the lingual aspect of the teeth (red line) reveal Sharpey fibres penetrating both mesial and distal facing bone surfaces and forming an "upright V" pattern within crestal bone. Sections cut more buccally (green line) reveal Sharpey fibres penetrating only the distal facing bone surface and the alveolar crest. The level of horizontal section is indicated by the blue line. The direction of tooth migration is arrowed. M1, first molar; M2, second molar; M, mesial; D, distal; Bu, buccal; L, lingual.

In addition, in buccolingual sections it was also possible to see Sharpey fibres penetrating both buccal and lingual facing bone surfaces. This was evident in sections cut through the mesial aspect of the teeth (Fig. 62). Sections examined farther distally through the tooth (Fig. 62) revealed Sharpey fibres penetrating only the buccal facing bone surface in a pattern of buccal tooth migration.

Confirmation of the histologic findings was provided by the incorporation of the radioactive label into forming bone matrix. The two administrations of tritiated proline produced labelled bone at the alveolar crest and along the distal and buccal facing bone surfaces in response to bone deposition (Figs. 42, 46). Distal, occlusal and buccal tooth migration was verified. Furthermore, the greater distance between the two silver grain lines at the alveolar crest suggested a higher rate of occlusal drift compared with the distal and buccal vectors. This had previously been reported by Hoffman and Schour (1940) who performed extensive quantitative studies using alizarin as the bone marker.

The radioautographic results of the present study differ from those of Tonna (1976) who reported a pattern of resorption on the distal facing surface of alveolar bone adjacent to the mesial root of the first molar. Although only the upper first molar was examined he believed that this pattern was formed in response to

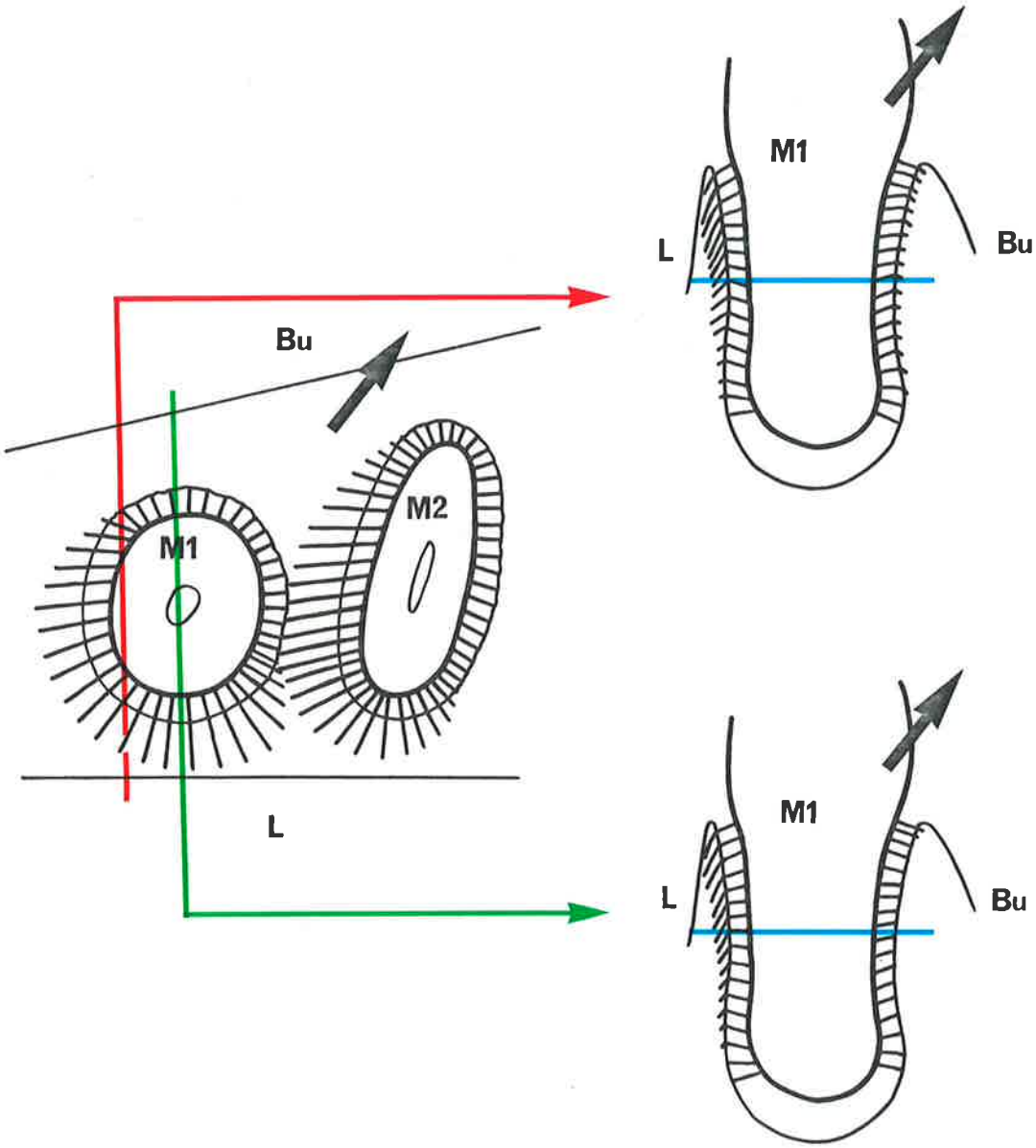


Figure 62. These schematic diagrams indicate the effect of the buccolingual plane of section on the relationship of Sharpey fibres with alveolar bone surfaces. Sections cut from the mesial aspect of the teeth (red line) reveal Sharpey fibres penetrating both buccal and lingual facing surfaces. Sections cut further distally (green line) reveal Sharpey fibres penetrating only the buccal facing bone surface. The level of horizontal section is indicated by the blue line. The direction of tooth migration is arrowed. M1, first molar; M2, second molar; Bu, buccal. L, lingual.

mesial tooth migration in his experimental mice. Kameyama (1973) similarly noted no radioautographic label along this surface in erupting teeth of three- and four-week old rats. The teeth examined were in pre-functional occlusion and considered to be tilting mesially. The present study found a line of silver grains along the distal facing surface adjacent to the mesial root of the first molar indicating that this surface was depository not resorptive in nature. The teeth examined by Tonna (1976) were possibly tilting mesially in accordance with Kameyama's (1973) description. The unusual finding of mesial tooth tilt accompanying distal migration in young rats had previously been recorded by Hoffman and Schour (1940).

Hoffman and Schour (1940) suggested that tilting was a major component of tooth movement. It was difficult to determine in the present study whether a tilting factor was present in any direction of migration. Although the line of silver grains revealed a decreasing amount of bone deposition from the crest to the apical region this may have been due to the occlusal component of drift exceeding that in other directions. In order to determine tooth tilting movements, Hoffman and Schour (1940) used linear measurements from the top of the alveolar crest to the cementoenamel junction of mesial and distal facing surfaces of adjacent teeth in rats up to five hundred days old. This procedure did not take into account any decrease in height of the alveolar crest due

to inflammatory or age changes which occur beyond two hundred days of age (Belting, Schour, Weinmann and Shepro, 1953). Similar measurements using material of the present study would not have been informative since the decalcification process had removed the tooth enamel and obscured the cementoenamel junction. Furthermore, variations in the plane of the histologic section made any such determinations, as well as any attempts to quantify bone deposition, extremely hazardous. The plane of tissue sectioning was greatly influenced by the orientation of the specimen when embedding and cutting despite attempts to standardize the technique. As well, rodent molars show an increasing lingual inclination from the first to third molar so that it is not possible to properly view all teeth in the same mid-sagittal plane (Fig. 3).

The present study has shown that large Sharpey fibres were associated with distal and buccal facing bone surfaces and also the alveolar crest. Silver grain distribution also corresponded to these particular bone surfaces. It was therefore concluded that use of Sharpey fibre patterns was a reliable method of histologically indicating sites of bone deposition and hence the direction of tooth migration.

TOOTH MIGRATION IN LATHYRISM.

The use of histologic and radioautographic indicators revealed a gradual alteration of tooth

migration during lathyrism. Since histologically the continuity of principal and Sharpey fibres into the distal facing surface of alveolar bone was disturbed it was concluded that these bone surfaces had changed their activity. Being depository in normal animals they were becoming resorptive surfaces in the pathological state of lathyrism. Furthermore, since the lathyritic disruption of the periodontal ligament was progressive, the change in activity of the distal facing surface appeared similarly progressive. Accompanying the resorptive changes in the distal facing bone surface, the buccal facing bone surface also exhibited a loss of Sharpey and principal fibre continuity.

In the present study the maximum amount of bone resorption was observed after forty days of lathyrism and accompanied the appearance of the lathyritic lesion. However, resorption was not seen to involve the alveolar crest where the regional periodontal fibres appeared intact. Accordingly, it was considered that a loss of integrity of the principal fibres of the periodontal ligament was related to the bone change. Also, these results suggested that the distal and buccal vectors of migration were primarily affected but perhaps not the occlusal vector.

Radioautographic studies related to lathyrism have attempted to determine the nature of the disease (Tanzer and Hunt, 1964; Tanzer and Gross, 1964). However,

the topographic distribution of radioactive amino acids within the periodontal ligament described by Kennedy and Kennedy (1963) bears marked similarities to the silver grain distribution in lathyritic animals seen in the present study. In severely lathyritic mice alternating light and dark bands of silver grains were found within the periodontal ligament. The heavy accumulations of label appeared within the amorphous lathyritic material adjacent to the pallisading cells, suggesting that cell utilization of proline during lathyrism was unaffected (Fig. 4).

The change in pattern of silver grain distribution within alveolar bone confirmed the histologic impression that the distal and buccal migration patterns had ceased. As expected, the first administration of proline labelled bone in a similar fashion to control animals and provided a baseline for further comparison (Fig. 51). Since bone deposition, as revealed by the second administration of proline, continued in its customary pattern during the early stages of lathyrism it indicated that the lathyritic effect on bone was not immediate. Bone deposition continued for some time before the first effects of lathyritic resorption were seen lateral to the root apices (Fig. 52). After forty days of lathyrism the finding that silver grains were not deposited at the distal and buccal facing surfaces, and the ultimate removal of the initial radioautographic marker, revealed the disturbed bone activity at these

surfaces. At this experimental period only bone deposition at the alveolar crest and at the base of the alveolus was continuing (Figs. 57, 58).

Since the lathyritic effect on bone was not immediate a relationship could be seen between the developing disturbance of the periodontal ligament and the induced bone changes. While the morphologic appearance of the periodontal ligament remained intact silver grains indicating bone deposition were found at the customary bone depository sites. This was exemplified in the ten-day lathyritic mouse in which the lathyritic periodontal ligament disruption was confined to the apical areas and the buccal and distal bone surface activity remained unchanged. At the end of forty days of experimental lathyrism the morphologic periodontal ligament disruption and bone resorption had, in concert, progressed up the periodontal ligament. This finding confirms the result of Kennedy and Kennedy (1963) who found no radioautographic label in bone surfaces adjacent to the lathyritic lesion. It was, therefore, considered that the morphologic disturbance of the periodontal ligament as a result of lathyrism was directly associated with the observed bone resorption. This further suggests that the integrity of the periodontal ligament is of importance in the maintenance of normal bone surface activity.

In a mesiodistal plane, the change in lathyritic bone activity seen in the present study confirmed

the findings of Sims (1977) who reported lathyritic bone resorption on both sides of the interdental septum. Similar observations were also reported by Gardner et al (1958) who stated that bone deposition had ceased after three weeks of lathyrism in rats, except at the alveolar crest where apposition was continuing. Although their findings were based purely on histologic criteria, the time period reported for the bone changes were comparable with the present study.

Resorption of both mesial and distal facing surfaces of the alveolar bone would suggest that the width of bone in the interdental and interradicular areas was decreasing. Although this was a general impression of the present study, the small sample size and the variation of section cutting provided no firm conclusion. However, Barrington and Myer (1966) did report substantial bone loss in these areas in lathyritic rats. Diastema formation between lathyritic rat molars reported by Sciaky and Ungar (1961) appeared to be at variance with the present and previous results. Although the severity of the induced lathyrism might be responsible, the appearance of diastemata would seemingly indicate an unusual drift pattern in which the first and third molars moved away from the second. Such tooth movement was not revealed by histologic and radioautographic evidence of bone activity. Furthermore, no obvious spaces occurred between the molar crowns.

TOOTH ERUPTION IN LATHYRISM.

The present study found substantial and continuing bone deposition occurring at the alveolar crest and at the base of the alveolus in forty-day lathyritic mice. Although quantification was not done, this finding places doubt over studies by Thomas (1965) and Michaeli et al (1975) who determined a marked retardation of tooth eruption in lathyritic rats. This led them to believe that an eruption force was generated by maturing collagen. As the stabilizing collagen cross-links formed, a tensional force was produced between tooth and bone. The observed continuance of mouse molar eruption in the presence of a lathyritically disturbed periodontal ligament might not support the collagen contraction theory of tooth eruption. This conclusion was previously expressed by Berkovitz et al (1972) who found continuing unimpeded incisor eruption in lathyritic rats. In addition, Bailey (1976) denied the causative role of collagen contraction on a molecular and structural basis. He argued that only lateral contraction of maturing collagen was possible which was therefore unable to produce a tensional eruption force vertically.

Apart from the choice of experimental animal (McCallum, 1965) and the difference in the lathyritic agents (Dasler and Milliser, 1957) both of which profoundly affect the results, a major discrepancy between the present and past studies was the choice of teeth examined. Previous studies examined the rodent incisor

which is a tooth of continual eruption and differs anatomically and physiologically from the molars (Matena, 1973). Furthermore, Sciaky and Ungar (1961) and the present study showed no morphologic disruption of the lathyritic incisor periodontal ligament compared with the molar.

Studies by Berkovitz et al (1972) and Sarnat and Sciaky (1965) revealed that it was the unimpeded tooth that continued to erupt during lathyrism while the impeded tooth was retarded. Although developmental studies by Cohn (1957) and Atkinson (1972) revealed that all mouse molars were in occlusion by at least six weeks of age, it is conceivable in the present study that these teeth were not contacting their antagonists for a number of reasons.

Firstly, it is possible that an unusual masticatory pattern existed in which the incisors were selectively used. Thus the occlusal load on the molars would have been relieved and unimpeded conditions created posteriorly.

Thomas (1976) believed that the consistency of the diet was of great importance. Hard coarse food by its very nature could excessively load teeth during mastication and act to impede tooth eruption. A fine soft diet as used in the present study may not have exerted such an effect.

Thirdly, it was suggested by Michaeli et al (1975) that rodent behavioural patterns might contribute to a variation in molar occlusion. Prolonged periods of mouth opening, during sleep or more likely during incisor gnawing activities, relieved the molar occlusion and created unimpeded eruption conditions. Valid though the above reasons may be, it was not possible to determine from the histologic evidence of the present study whether the mouse molars were out of opposing contact, although Krikos et al (1965) determined that the lathyritic disturbance only occurred in the presence of occlusal stress.

It has been revealed in the present study that the effects of lathyrism were gradual. The reversal of distal and buccal migration was not immediately evident since bone deposition on distal and buccal facing surfaces continued for some time after the initiation of the disease. Accordingly, a similar progressive alteration in tooth eruption may occur but over a longer period of time. In this respect a more severe induction or a longer lathyritic period may be required for these changes to become apparent.

The possibility that collagen is not actively involved in tooth eruption raises the question of other mechanisms being responsible. Ness (1967) suggested that tissue fluid pressure was of importance. In this regard the increased vascularity seen in the lathyritic periodontal ligament may be of some relevance.

Recently, attention has been drawn to the contractibility of fibroblasts after Ness (1967) suggested that these cells in the periodontal ligament acted as the prime mover in tooth eruption. Beertsen, Everts and van den Hooff (1974) electron microscopically examined incisor and molar periodontal ligament fibroblasts of rats. They reported that incisor fibroblasts contained microfilaments and microtubules which were thought to be intracellular signs indicating cellular locomotion (Goldman, 1971). Beertsen et al (1974) considered that, as these cells migrated within the periodontal ligament, they produced an axial pull on the tooth using the network of collagen fibres. In distinction, molar periodontal ligament fibroblasts contained fewer micro-structures which posed a likely explanation for the limited eruption of molars compared with the continuous eruption of rodent incisor teeth. However, an ultrastructural study by Shore and Berkovitz (1979) found no morphological differences between incisor and periodontal ligament fibroblasts.

At the light microscopic level of the present study the cells of the lathyritic molar periodontal ligament increased in number and size, became more basophilic and aligned occlusoapically. Although the present author knows of no electron microscopic studies of lathyritic periodontal fibroblasts, it is tempting to think that the observed increase in cellular activity is in some way related to the continuing molar eruption.

Opposing the concepts of Ness (1967) and Beertsen et al (1974), Shore and Berkovitz (1979) reported no specific orientation of cells in the normal rat molar periodontal ligament and did not consider that fibroblasts generated an eruptive force. Garant and Cho (1979b) related fibroblast orientation and polarization to cell migration and collagen secretion in rodents. The occlusoapical orientation of lathyritic fibroblasts may be a result of the continuing tooth eruption rather than being actively involved.

The change in fibroblast number and staining characteristics may be a result of an alteration of cell function induced by lathyrism. Ten Cate, Deporter and Freeman (1976) showed that fibroblasts possessed the ability to produce and degrade collagen and so were intimately involved with its turnover. Since collagen synthesis is not disturbed (Gerber et al, 1962; Fry et al, 1962; Tanzer and Gross, 1964) increased cellular efforts to remove the defective lathyritic collagen possibly accounts for the cell transformation.

THE TRANSSEPTAL FIBRES IN TOOTH MIGRATION.

Picton and Moss (1973) and Moss and Picton (1974) believed that the transseptal fibre system linking adjacent teeth was the principal aetiologic factor in approximal tooth migration. Mechanically interfering with the integrity of the transseptal fibre chain in monkeys greatly reduced the amount of tooth drift.

Although the disease of lathyrism morphologically altered the principal fibres of the periodontal ligament the present study revealed that forty days of lathyrism seemingly did not disturb the transseptal group (Fig. 10). However, histologic and radioautographic indicators disclosed an altered pattern of tooth migration. The cessation of the distal and buccal vectors of tooth migration and the intact nature of the transseptal fibres appear inconsistent with Moss' and Picton's concept. Furthermore, it is difficult to relate the orientation of the transseptal group to the buccal component of mouse molar migration. Although the transseptal fibres may be altered at a physical, chemical, biochemical or other higher level, it may well be that the principal fibres of the periodontal ligament play a greater role in tooth migration than previously believed, particularly in a distal and buccal direction since they were the fibres principally affected by lathyrism.

Moss and Picton (1974) have shown the involvement of the transseptal fibres in tooth migration, and the present study has implicated the principal fibres of the periodontal ligament. It must be appreciated that the dentition of rodents differs anatomically and possibly functionally from the dentition of monkeys. Nevertheless, it may be erroneous to single out a particular fibre system as being solely responsible for tooth migration. It is possible that the integrity of the entire periodontal ligament is essential for tooth movement whether in a

direct causative role or indirectly in response to other external forces.

COLLAGEN UNDER STRESS.

Hamre and Yeagre (1957) reported exostosis formation at the site of muscle insertions in lathyritic rats. It was believed that muscle stress on the weakened periosteum caused its detachment from bone and resulted in bone deposition. Similarly, occlusal stress on a weakened periodontium was associated with the morphologic changes in the periodontal ligament and alveolar bone (Krikos et al, 1965). However, orthodontic stress applied to the lathyritic periodontal ligament resulted in no disturbance of the ligament fibres and a higher than normal rate of alveolar bone formation (Heller and Nanda, 1979). These conflicting results indicate that the behaviour of lathyritic collagen under stress is incompletely understood.

The present study did not specifically examine occlusal stress as it applied to the morphologic changes in the lathyritic periodontal ligament. Just as it is difficult to relate the present findings to those of Krikos et al (1965) and Heller and Nanda (1979), it is also difficult to account for the differing lathyritic effects in the molar and incisor periodontal ligaments. Both ligaments had a high turnover of collagen and presumably were subject to masticatory load, yet the

amorphous lathyritic masses were only seen surrounding the molars. The continuing eruption of molars during the experimental period of the present study suggests that the behaviour of lathyritic collagen under stress is inconsistent and an area requiring further research, particularly with respect to occlusal loading of a lathyritic periodontal ligament.

CHAPTER 7

CONCLUSIONS.

1. A histologic examination of principal fibres of the periodontal ligament revealed size variations between fibres related to mesial and distal facing bone surfaces. Similar size variations were apparent in Sharpey fibres associated with these surfaces.

2. The deep penetration of Sharpey fibres into the alveolar crest and distal facing bone surface was taken to reflect occlusal and distal tooth migration. Moreover, a third vector of buccal migration was indicated.

3. No evidence supporting a transalveolar tooth-to-tooth fibre system could be found. Sharpey fibres crossing the alveolar septa terminated either at resting lines within bone or at mesial facing resorptive bone surfaces. A V-pattern of Sharpey fibres previously described within the alveolar septa was only observed near the lingual aspect of the teeth and related to the buccal component of tooth migration.

4. Radioautographic confirmation of the histologic signs of tooth migration was provided by lines of silver grains which followed the contour of the alveolar crest and the distal and buccal facing bone surfaces. The drift pattern remained constant in control animals although the distance between the two lines of label at the alveolar crest was greater than the distance between the lines in other areas. This suggested a higher rate of occlusal drift.

5. Deeply penetrating Sharpey fibres were related to areas of bone where silver grain label had been deposited. It was concluded that Sharpey fibres were reliable histologic indicators of tooth migration.

6. A diet of 50% sweet pea appeared adequate to produce the microscopic signs of lathyrism even though the macroscopic signs were not evident after forty days.

7. The lathyritic change within the periodontal ligament was seen to progress from the apical to the crestal region but failed to involve the transseptal fibre group. This distribution of lathyritic change was related to the turnover of collagen within the periodontal ligament.

8. Lathyrism destroyed Sharpey and principal fibre continuity at the alveolar bone-periodontal ligament interface. This was evident within the periodontal ligament opposite the lathyritic lesion rather than at the alveolar crest and suggested that tooth migration had ceased in the distal and buccal directions.

9. The lathyritic bone changes were likely explained by the inability of a weakened periodontal ligament to support the tooth against occlusal load.

10. Silver grain distribution in radioautographs of lathyritic tissue sections confirmed the histologic impression of an altered tooth migration pattern. Silver grains were not deposited in bone adjacent to the lathyritic lesion, but were present at the alveolar crest. It was apparent that distal and buccal migration had ceased yet occlusal drift was continuing.

11. The continuing occlusal drift was related to possible unimpeded molar conditions, to possible cellular effects, or to delayed effects of lathyrism on eruption. Nevertheless, the collagen contraction theory of tooth eruption becomes untenable.

12. The morphologically intact nature of the transseptal fibre system in the presence of an altered pattern of tooth migration questioned their primary role in approximal tooth drift. The disturbance of the principal fibres of the periodontal ligament indicated that they might play a key role in tooth migration. It was suggested that the integrity of the entire periodontal ligament was essential for tooth migration.

13. The differing effects of lathyrism on the incisor and molar periodontal ligaments and the incompletely understood behaviour of lathyritic collagen under stress were suggested as areas requiring further research. In addition, throughout the investigation the present author was impressed by the lack of information at the light microscope and other levels concerning the cellular changes that occur during lathyrism. Studies examining the possible influence of the normal and lathyritic fibroblast on tooth migration may also be fruitful.

CHAPTER 8

APPENDIX 1.

RADIOCHEMICAL SPECIFICATIONS.

Radioactive proline - NET-090 PROLINE,
L-{3,4-^{3}H(N)}
NEW ENGLAND NUCLEAR.

SPECIFIC LOT DATA.

Lot number	:	1070 - 109
Specific activity	:	25.0 Ci/mmol.
Concentration	:	5.0mCi; 0.023mg in 5.0ml (0.01 N HCl) 1.0 Ci/L.
Purity	:	98.5% tested on 11.9.77. 98.0% tested on 29.1.79.

DILUTION FOR INJECTION.

A 25% dilution of L-{3,4,-^{3}H(N)} proline stock

$= 25 \times 10^{-2} \times 1$ Ci/L.

= 250μCi/ml.

Average mouse weight = 20 grams

* at 5μCi/gram body weight
total dose equals 100μCi.

Average injected dose in mls. $= \frac{100}{250}$ mls.

$= .40$ ml.

Each mouse was weighed and the dosage adjusted accordingly.

APPENDIX 2.

BOUIN'S SOLUTION (Luna, 1968, p.5).

Picric acid, saturated aqueous solution:	750.0 ml.
37-40% formalin:	250.0 ml.
Glacial acetic acid:	50.0 ml.

APPENDIX 3.

TISSUE PROCESSING FOR LIGHT MICROSCOPY.

The technique of Peterfi. (Culling, 1974).

The specimens were subjected to the following procedure at a constant temperature of 37°C.

1.	70% alcohol	...	overnight
2.	80% alcohol	...	1 hour
3.	90% alcohol	...	1 hour
4.	95% alcohol	...	1 hour
5.	Absolute alcohol	...	1 hour
6.	Absolute alcohol	...	1 hour
7.	Absolute alcohol	...	1 hour

The tissues were then infused with clean paraffin wax at a constant temperature of 56°C.

1.	2/3 methyl salicylate 1/3 paraffin wax	...	1 hour
2.	1/2 methyl salicylate 1/2 paraffin wax	...	1 hour
3.	1/3 methyl salicylate 2/3 paraffin wax	...	1 hour
4.	Paraffin wax (first change)	...	2 hours
5.	Paraffin wax (second change)	...	2 hours
6.	Paraffin wax (third change)	...	overnight

The tissues were then vacuumed in paraffin wax at a temperature of 56°C for approximately 15 minutes prior to embedding in blocks.

CHAPTER 9

BIBLIOGRAPHY.

Atkinson M.E., 1972. The development of the mouse molar periodontium. Journal of Periodontal Research, 7, 255-260.

Bailey A.J., 1976. In: The Eruption and Occlusion of Teeth (edited by Poole G.H.J. and Stack M.V.) pp. 305-306. Colston Papers No. 27, Butterworths, London.

Baron R., 1973. Remaniement de l'os alveolaire et des fibre desmodontales au course de la migration physiologique. (Remodelling of alveolar bone and periodontal ligament during physiologic drift.) Journal de Biologie Buccale, 1, 151-170.

Barrington E.P. and Meyer J., 1966. Recovery of the rat dental organ from experimental lathyrism. Journal of Periodontology, 37, 453-467.

Barrow M.V., Simpson C.F. and Miller E.J., 1974. Lathyrism: A Review. The Quarterly Review of Biology, 49, 101-128.

Baume L.J., 1950. Physiologic tooth migration and its significance for the development of occlusion. III. The biogenesis of the successional dentition. Journal of Dental Research, 29: 338-348.

Baumrind S., 1969. A reconsideration of the propriety of the "pressure-tension" hypothesis. American Journal of Orthodontics, 55:12-22.

Beertsen W., Everts V. and van den Hooff A., 1974. Fine structure of fibroblasts in the periodontal ligament of the rat incisor and their possible role in tooth eruption. Archives of oral Biology, 19:1087-1098.

Beertsen W. and Tonino G.J.M., 1975. Effects of fixation and demineralization on the intensity of autoradiographic labelling over the periodontal ligament of the mouse incisor after administration of {^{3}H}-proline. Archives of oral Biology, 20, 189-193.

Belanger L.F., 1961. Staining processed radioautographs. Stain Technology, 36:313-317.

Bell E.A. and O'Donovan J.P., 1966. The isolation of α & γ -oxalyl derivatives of α,γ-diaminobutyric acid from seeds of Lathyrus latifolius and the detection of the α-oxalyl isomer of the neurotoxin α-amino-β-oxalyl aminopropionic acid which occurs together with the neurotoxin in this and other species. Phytochemistry, 5:1211-1219.

Belting C.M., Schour I., Weinmann J.P. and Shepro M.J., 1953. Age changes in the periodontal tissues of the rat molar. Journal of Dental Research, 32:332-353.

Berkovitz B.K.B., 1971. The effect of root transection and partial root resection on the unimpeded eruption rate of the rat incisor. Archives of oral Biology, 16:1033-1043.

Berkovitz B.K.B., 1974. The effect of a vitamin C deficient diet on eruption rates for the guinea-pig lower incisor. Archives of oral Biology, 19:807-812.

Berkovitz B.K.B. and Thomas N.R., 1969. Unimpeded eruption in the root-resected lower incisor of the rat with a preliminary note on root transection. Archives of oral Biology, 14:771-780.

Berkovitz B.K.B., Migdalski A. and Solomon M., 1972. The effect of the lathyritic agent aminoacetonitrile on the unimpeded eruption rate in normal and root-resected rat lower incisors. Archives of oral Biology, 17:1755-1763.

Bernick S., 1960. The organization of the periodontal membrane fibres of the developing molars of rats. Archives of oral Biology, 2, 57-63.

Bernick S., Levy B.M., Dreizen S. and Grant D.A., 1977. The intraosseous orientation of the alveolar component of marmoset alveolodental fibres. Journal of Dental Research, 56:1409-1416.

Bevelander G., 1964. The effect of tetracycline on mineralization and growth. Advances in Oral Biology, 1:205-223.

Black G.V., 1886. Periosteum and peridental membrane. Dental Review (Wien), 1:233-243, 289-302.

Boren H.G., Wright E.C. and Harris C.C., 1975. Quantitative light microscopic autoradiography emulsion sensitivity and latent image fading. Journal of Histochemistry and Cytochemistry, 23, 901-909.

Bornstein P., 1970. The cross-linking of collagen and elastin and its inhibition in osteolathyrism. Is there a relation to the aging process? The American Journal of Medicine, 49, 429-435.

Brash, J.C., 1926. The growth of the alveolar bone and its relation to the movements of the teeth, including eruption. Dental Record, 46:641-664; 47:1-26; 175-186.

Burnett G.J., 1978. An autoradiographic and developmental study of transalveolar fibres in the mouse mandible. M.D.S. Thesis, The University of Adelaide.

Carmichael D.J. and Dodd C.M., 1974. Studies on matrix proteins of normal and lathyritic rat bone and dentine. Calcified Tissue Research, 14:177-194.

Carneiro J., 1965. Synthesis and turnover of collagen in periodontal tissues. International Society of Cellular Biology Symposium, 4, 247-257.

Carneiro J. and Fava de Moraes F., 1965. Radioautographic visualization of collagen metabolism in the periodontal tissues of the mouse. Archives of oral Biology, 10, 833-848.

Carneiro J. and Leblond C.P., 1966. Suitability of collagenase treatment for the radioautographic identification of newly synthesized collagen labelled with ^{3}H-glycine or ^{3}H-proline. Journal of Histochemistry and Cytochemistry, 14, 334-344.

Cason J.E., 1950. A rapid one-step Mallory-Heidenhain stain for connective tissue. Stain Technology, 50:225-226.

Cleall J.F., Perkins R.E. and Gilda J.E., 1964. Bone marking agents for the longitudinal study of growth in animals. Archives of oral Biology, 9:627-646.

Cohn S.A., 1957. Development of the molar teeth in the albino mouse. American Journal of Anatomy, 101:295-320.

Cohn S.A., 1965. Disuse atrophy of the periodontium in mice. Archives of oral Biology, 10:909-919.

Cohn S.A., 1972a. A re-examination of Sharpey's fibres in alveolar bone of the mouse. Archives of oral Biology, 17, 255-259.

Cohn S.A., 1972b. A re-examination of Sharpey fibres in alveolar bone of the marmoset (Sanguinus fusicollis). Archives of oral Biology, 17, 261-269.

Cohn S.A., 1973. Transalveolar fibres in the teeth of Macaca mulatta. Journal of Dental Research, 52, 155 (Abstract).

Cohn S.A., 1975. Transalveolar fibres in the human periodontium. Archives of oral Biology, 20, 257-259.

Craik J.A. and McNeil I.R.R., 1966. Micro-architecture of skin and its behaviour under stress. Nature, 209, 931-932.

Crumley P.J., 1964. Collagen formation in the normal and stressed periodontium. Periodontics, 2, 53-61.

Culling C.F.A., 1974. Handbook of histopathological and histochemical techniques. 3rd Edition, Butterworth & Co., London.

Dasler W., 1954. Isolation of toxic crystals from sweet pea (Lathyrus odoratus). Science, 120, 307-308.

Dasler W. and Milliser R.V., 1957. Experimental lathyrism in mice fed diets containing sweet peas or β-aminopropionitrile. Proceedings of the Society of Experimental Biology and Medicine, 96, 171-174.

Diaz E.A., 1978. Periodontal ligament collagen response to tooth movement: histochemical and autoradiographic reactions. American Journal of Orthodontics, 73, 443-458.

Dunstan I.H., 1975. Collagen fibre patterns in the alveolar process of the mouse mandible. B. Sc. Dent. Thesis, The University of Adelaide.

Dupuy H.P. and Lee J.G., 1954. The isolation of a material capable of producing experimental lathyrism. Journal of the American Pharmacology Association (Scient. Ed.), 43:61.

Duterloo H.S. and Bierman M.W.J., 1976. Structural changes in alveolar bone during the development of the dentition. Transactions of the European Orthodontic Society, 52nd Congress, pages 177-183.

Eastoe J.E., 1976. Collagen chemistry and tissue organisation. In: The Eruption and Occlusion of Teeth (Edited by Poole D.F.G. and Stack M.V.), pp. 229-251. Colston Papers No. 27. Butterworths, London.

Edwards R.J., 1977. Perforating fibres in the human mandible. M.D.S. Thesis, University of Adelaide.

Formicola A.J. and Ferrigno P.D., 1966. A radioautographic study of the developing periodontium of the rat. Periodontics, 4, 297-301.

Fry P., Harkness M.L.R., Harkness R.D. and Nightingale M., 1962. Mechanical properties of lathyritic animals. Journal of Physiology, 164, 77-89.

Garant P.R. and Cho M., 1979a. Autoradiographic evidence of the coordination of the genesis of Sharpey's fibres with new bone formation in the periodontium of the mouse. Journal of Periodontal Research, 14, 107-114.

Garant P.R. and Cho M., 1979b. Cytoplasmic polarization of periodontal ligament fibroblasts. Implications for cell migration and collagen secretion. Journal of Periodontal Research, 14:95-106.

Gardner A.F., 1959a. Experimental lathyrism: review of the literature. American Journal of Clinical Nutrition, 7, 213-233.

Gardner A.F., 1959b. Influence of injury and adaptation of the periodontal ligament to pathological changes during experimental lathyrism. Journal of Periodontology, 30, 253-264.

Gardner A.F., 1960. Morphological study of oral connective tissues in lathyrism. Journal of Dental Research, 39, 24-45.

Gardner A.F., Dasler W. and Weinmann, J.P., 1958. Masticatory apparatus of the albino rat in experimental lathyrism. Journal of Dental Research, 37, 492-515.

Geiger B.J., Steenbock H. and Parsons H.T., 1933. Lathyrism in the rat. Journal of Nutrition, 6, 427-442.

Gerber G.B., Gerber G. and Altman K.I., 1962. Studies of collagen turnover in lathyritic rats. Archives of Biochemistry and Biophysics, 96, 601-604.

Gianelly A.A. and Goldman H.M., 1971. Biological basis of orthodontics. Lea and Febiger, Philadelphia.

Goldman H.M., 1957. The behaviour of transseptal fibres in periodontal disease. Journal of Dental Research, 36, 249-254.

Goldman R.D., 1971. The role of three cytoplasmic fibres in BHK-21 cell motility - I: Microtubules and the effect of colchicine. Journal of Cell Biology, 51:752-762.

Goren A.D., Singh I.J. and Pentel A.N., 1977. The effects of induced lathyrism on Sr^{85} uptake in rats during late prenatal development. American Journal of Anatomy, 150:193-199.

Grant D.A., Stearn I.B. and Everett F.G., 1968. Orban's periodontics. Third edition. The C.V. Mosby Co., St. Louis.

Grant G. and Bernick S., 1972. Formation of the periodontal ligament. Journal of Periodontology, 43:17-25.

Grimstone A.V. and Skaer R.J., 1972. A guidebook to microscopical methods. Cambridge University Press.

Gross J., 1963a. An intermolecular defect of collagen in experimental lathyrism. Biochimica et Biophysica Acta., 71, 250-252.

Gross J., 1963b. An intermolecular defect of collagen in experimental lathyrism. Biochimica et Biophysica Acta., 74, 314-316.

Ham A.W., 1965. Histology. 5th Edition. J.B. Lippincott Co., Philadelphia and Montreal.

Hamre C.J. and Yaeger V.L., 1957. Influence of muscle section on exostoses of lathyritic rats. Archives of Pathology, 64, 426-433.

Hancox N.M., 1972. Biology of Bone. Cambridge University Press.

Hausmann E. and Neumann W.F., 1961. Conversion of proline to hydroxyproline and its incorporation into collagen. Journal of Biological Chemistry, 236, 149-152.

Heller I.J. and Nanda R., 1979. Effect of metabolic alteration of periodontal fibres on orthodontic tooth movement. An experimental study. American Journal of Orthodontics, 75, 239-258.

Herovici C., 1963. A polychrome stain for differentiating precollagen from collagen. Stain Technology, 38, 204-205.

Hindle M.O., 1967. The intermediate plexus of the periodontal ligament. In: The mechanisms of tooth support. A symposium. pp. 66-71. John Wright and Sons Limited, Bristol.

Hoffman M.M. and Schour I., 1940. Quantitative studies in the development of the rat molar: II. Alveolar bone, cementum, and eruption. American Journal of Orthodontics and Oral Surgery, 26:854-874.

Hoyte D.A.N., 1960. Alizarin as an indicator of bone growth. Journal of Anatomy, 94:432-442.

Hoyte D.A.N., 1968. Alizarin red in the study of the apposition and resorption of bone. American Journal of Physical Anthropology, 29:157-178.

Hurley J.V. and Ham J.N., 1959. The nature of the connective tissue defect produced by the aminonitriles. British Journal of Experimental Pathology, 40, 216-224.

Jackson S.A. and Heininger J.A., 1974. A study of collagen neutilization using an O^{18} labelling technique. Clinica Chemica Acta, 51, 163-171.

Johnson R.B., 1980. Development of transalveolar fibres in the mouse periodontium. Anatomical Record, 196:90A (abstract).

Johnson R.H. and Mitchell D.F., 1966. The effects of tetracyclines on teeth and bones. Journal of Dental Research, 45:86-93.

Kameyama Y., 1973. The pattern of alveolar bone activity during development and eruption of the molar in the rat. Journal of Periodontal Research, 8:179-191.

Kameyama Y., 1975. Autoradiographic study of ^{3}H-proline incorporation by rat periodontal ligament, gingival connective tissue and dental pulp. Journal of Periodontal Research, 10, 98-102.

Kardos T.B. and Simpson L.O., 1980. A new membrane biology based on thixotrophic concepts. American Journal of Orthodontics, 77:508-515.

Kennedy J.S. and Kennedy G.D.C., 1962. Autoradiographic studies in experimental lathyrism. Journal of Pathology and Bacteriology, 84, 123-134.

Kennedy G.D.C. and Kennedy J.S., 1963. An autoradiographic study of the dental lesions of lathyrism in the rat. Archives of oral Biology, 8, 207-217.

Kraw A.G. and Enlow H.E., 1967. Continuous attachment of the periodontal membrane. American Journal of Anatomy, 120, 133-142.

Krikos G.A., 1964. Histochemical studies of the periodontal ligament in lathyritic rats. Archives of oral Biology, 9:415-420.

Krikos G.A., Morris A.L., Hammond W.S. and McClure H.H., 1958. Oral changes in experimental lathyrism (odoratism). Oral Surgery, Oral Medicine and Oral Pathology, 11, 309-322.

Krikos G.A., Beltran R. and Cohan A., 1965. Significance of mechanical stress on the development of periodontal lesions in lathyritic rats. Journal of Dental Research, 44, 600-607.

Krutchkoff D., 1972. Radioautography: A useful technique in dental research. Periodontal Abstracts, Vol. XX, No. 1, pp. 11-15.

Kurihara S. and Enlow D.H., 1980. An electron microscope study of attachments between periodontal and fibres and bone during alveolar remodeling. American Journal of Orthodontics, 77:516-531.

Lacroix P., 1960. Ca^{45} autoradiography in the study of bone tissue. In: Bone as a tissue. Rodahl K., Nicholson J. and Brown E., editors. McGraw-Hill Book Company.

Leblond C.P., Wilkinson G.W., Belanger L.F. and Robichon J., 1950. Radioautographic visualization of bone formation in the rat. American Journal of Anatomy, 86:289-342.

Levene C.I., 1967. Experimental Osteolathyrism. In: The Connective Tissue. ed. Wagner B.M. and Smith D.E. The Williams and Wilkins Company, Baltimore, pp. 132-141.

Levene C.I., 1973. Lathyrism. In: Molecular Pathology of Connective Tissues, pages 175-228. Perez-Tamayo R. and Rojkind M. (Eds.). Dekker, New York.

Levene C.I. and Gross J., 1959. Alterations in state of molecular aggregation of collagen induced in chick embryos by β-aminopropionitrile (lathyrus factor). Journal of Experimental Medicine, 110, 771-787.

Levy G.G. and Maillard M.L., 1980. Histologic study of the effects of occlusal hypofunction following antagonist tooth extraction in the rat. Journal of Periodontology, 51:393-399.

Lillie R.D., 1965. Histopathologic technique and practical histochemistry. Third Edition. McGraw-Hill, Inc.

Luna L.G. (editor), 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. Third edition. American Registry of Pathology, McGraw-Hill.

McCallum H.M., 1965. Experimental lathyrism in mice. Journal of Pathology and Bacteriology, 89, 625-636.

Martin G.R., Gross J., Piez K.A. and Lewis M.S., 1961. On the intermolecular cross-linking of collagen in lathyritic rats. Biochemica et Biophysica Acta., 53, 599-601.

Matena V., 1973. Periodontal ligament of a rat incisor tooth. Journal of Periodontology, 44:629-636.

Melcher A.H. and Bowen W.H., 1969. Biology of the periodontium. Academic Press, London.

Menzies D.W. and Mills K.W., 1957. The aortic and skeletal lesions of lathyrism in rats on a diet of sweet pea. Journal of Pathology and Bacteriology, 73, 223-237.

Michaeli Y., Pitaru S., Zajicek G. and Weinreb M.M., 1975. Role of attrition and occlusal contact in the physiology of the rat incisor: IX. Impeded and unimpeded eruption in lathyritic rats. Journal of Dental Research, 54:891-896.

Mortreuil-Langlois M., 1962. Staining sections coated with radiographic emulsion; a nuclear fast red, indigo-carmine sequence. Stain Technology, 37, 175-177.

Moss J.P., 1976. Theories of tooth migration. In: The Eruption and Occlusion of Teeth (edited by Poole D.F.G. and Stack M.V.) Colston Papers No. 27, pp. 205-212. Butterworths, London.

Moss J.P. and Gray S.J., 1973. Serial sections of the jaw of the ferret. Laboratory Investigation, January, pp. 39-42.

Moss J.P. and Picton D.C.A., 1974. The effect on approximal drift of cheek teeth of dividing mandibular molars of adult monkeys (Macaca irus). Archives of oral Biology, 19, 1211-1214.

Ness A.R., 1967. Eruption - A review. In: The mechanisms of tooth support. Asymposium. pp. 84-88. John Wright and Sons, Bristol.

Norris W.P. and Woodruff L.A., 1955. The fundamentals of radioautography. Annual Review of Nuclear Science, 5, 297-326.

Noyes F.B., 1953. Oral histology and embryology. 7th edition edited and revised by Isaac Shour. Philadelphia, Lea and Febiger.

Orban B.J., 1976. Oral histology and embryology. 8th edition edited by G.S.N. Bhaskar, St. Louis, C.V. Mosby Co.

Orloff S.G. and Gross J., 1963. Experimental lathyrism in the chick embryo. The distribution of β-aminopropionitrile. Journal of Experimental Medicine, 117, 1009-1018.

Orlowski W.A., 1976. The incorporation of H^3-proline into the collagen of the periodontium of the rat. Journal of Periodontal Research, 11:96-100.

Page R.C. and Benditt E.P., 1972. Diseases of connective and vascular tissues. IV. The molecular basis for lathyrism. Laboratory Investigation, 26:22-26.

Picton D.C.A. and Moss J.P., 1973. The part played by the transseptal fibre system in experimental approximal drift of the cheek teeth of monkeys (Macaca irus). Archives of oral Biology, 18, 669-680.

Piez K.A., 1968. Crosslinking of collagen and elastin. Annual Review of Biochemistry, 37, 547-570.

Pinnell S.R. and Martin G.R., 1968. Cross-linking of collagen and elastin. The enzymatic conversion of lysine in peptide linkage to alpha-aminoadipic-5-semialdehyde by an extract from bone. Proceedings of the National Academy of Science of the United States, 61, 708-716.

Ponseti I.V., Wawzonek R.S., Shepard R.S., Evans T.C. and Stearns G., 1956. Further studies on lathyrism in the rat. Proceedings of the Society for Experimental Biology and Medicine, 92, 366-369.

Prescott G.H., Mitchell D.F. and Fahmy H., 1968. Procion dyes as matrix markers in growing bone and teeth. American Journal of Physical Anthropology, 29:219-224.

Quigley M.B., 1970. Perforating (Sharpey's) fibers of the periodontal ligament and bone. Alabama Journal of Medical Science, 7, 336-342.

Reddi A.H. and Sullivan N.E., 1979. Inhibition of mineralization by experimental lathyrism during matrix-induced endochondral bone differentiation. Proceedings of the Society for Experimental Biology and Medicine, 162:445-448.

Ressler C., 1962. Isolation and identification from the common vetch of the neurotoxin beta-cyano-L-alanine, a possible factor in neurolathyrism. Journal of Biological Chemistry, 237, 733-735.

Ressler C., Redstone P.A. and Erenberg R.H., 1961. Isolation and identification of a neuroactive factor from Lathyrus latifolius. Science, 134, 188-190.

Rippin J.W., 1976. Collagen turnover in the periodontal ligament under normal and altered functional forces. I. Young rat molars. Journal of Periodontal Research, 11, 101-107.

Rippin J.W., 1978. Collagen turnover in the periodontal ligament under normal and altered functional forces. II. Adult rat molars. Journal of Periodontal Research, 13, 149-154.

Ritchey B., and Orban B., 1953. Crests of the alveolar dental septa. Journal of Periodontology, 24, 75-87.

Rogers A.W., 1979. Techniques of Autoradiography. Second edition. Elsevier Scientific Publishing Co., Amsterdam.

Ross R. and Bendit E.P., 1965. Wound healing and collagen formation. Journal of Cell Biology, 27, 83-106.

Sarnat H. and Sciaky I., 1965. Experimental lathyrism in rats: effects of removing incisal stress. Periodontics, 3, 128-134.

Sciaky I. and Ungar H., 1961. Effects of experimental lathyrism on the suspensory apparatus of incisors and molars in rats. Annals of Dentistry (Balt.), 20:90-99.

Scott J.H., 1968. The development, structure and function of alveolar bone. Dental Practitioner, 19, 19-22.

Scott J.H. and Symons N.B.B., 1974. Introduction to dental anatomy. 7th Edition. Churchill Livingstone, Edinburgh and London.

Selye H., 1957. Lathyrism. Revue Canadienne de Biologie, 16, 1-82.

Shackleford J.M., 1971. The indifferent fibre plexus and its relationship to principal fibres of the periodontium. American Journal of Anatomy, 131: 427-441.

Shore R.C. and Berkovitz B.K.B., 1979. An ultrastructural study of periodontal ligament fibroblasts in relation to their possible role in tooth eruption and intracellular collagen degradation in the rat. Archives of oral Biology, 24:155-164.

Sicher H., 1923. Bau and funktion des fixations apparates der meerschwein chenmolaren. Zeitschrift fur Stomatologie, 21, 580-595.

Sicher H. and Weinmann J.P., 1944. Bone growth and physiologic tooth movement. American Journal of Orthodontics and Oral Surgery, 30, 109-132.

Sims M.R., 1977. The oxytalan fibre system in the mandibular periodontal ligament of the lathyritic mouse. Journal of oral Pathology, 6, 233-250.

Skougaard M.R., Levy B.M. and Simpson J., 1970. Collagen metabolism in skin and periodontal membrane of the marmoset. Scandinavian Journal of Dental Research, 78:256-262.

Skougaard M.R. and Levy B.M., 1971. Collagen metabolism in periodontal membrane of the marmoset, influence of periodontal disease. Scandinavian Journal of Dental Research, 79, 518-522.

Sodek J., 1976. A new approach to assessing collagen turnover by using a micro-assay. A highly efficient and rapid turnover of collagen in rat periodontal tissues. Journal of Biochemistry, 160, 243-246.

Stahl S.S. and Tonna E.A., 1977. H^3-proline study of aging periodontal ligament matrix formation: comparison between matrices adjacent to either cemental or bone surfaces. Journal of Periodontal Research, 12, 318-322.

Stallard R.E., 1963. The utilization of H^3-proline by the connective tissue elements of the periodontium. Periodontics, 1, 185-188.

Stamler F.W., 1955. Reproduction in rats fed lathyrus peas or aminonitriles. Proceedings of the Society for Experimental Biology and Medicine, 90, 294-298.

Stein G. and Weinmann J., 1925. Die physiologische wanderung der zahn. Zeitschrift fur Stomatologie, 23, 733-744.

Storey E., 1972. Growth and remodelling of bone and bones. American Journal of Orthodontics, 62, 142-165.

Tanzer M.L., 1965. Experimental lathyrism. In: International Review of Connective Tissue Research, Volume 3, pp. 91-112. Hall D.A. (Ed.), Academic Press, London.

Tanzer M.L., 1973. Cross-linking of collagen. Science, 180:561-566.

Tanzer M.L. and Gross J., 1964. Collagen metabolism in the normal and lathyritic chick. Journal of Experimental Medicine, 119, 275-289.

Tanzer M.L. and Hunt R.D., 1964. Experimental lathyrism. An autoradiographic study. Journal of Cell Biology, 22, 623-631.

Ten Cate A.R., Deporter D.A. and Freeman E., 1976. The role of fibroblasts in the remodeling of periodontal ligament during physiologic tooth movement. American Journal of Orthodontics, 69:155-168.

Thomas N.R., 1965. The effect of inhibition of collagen maturation on eruption in rats. Journal of Dental Research (Supplement), 44:1159 (abstract).

Thomas N.R., 1967. The properties of collagen in the periodontium. In: The mechanisms of tooth support. A symposium. pp. 102-106. John Wright and Sons Ltd., Bristol.

Thomas N.R., 1976. Collagen as the generator of tooth eruption. In: The eruption and occlusion of teeth. Poole D.F.G. and Stack M.V. (Eds.). Colston Papers No. 27. Butterworths, London.

Thurston J.M. and Joftes D.L., 1963. Stains compatible with dipping radioautography. Stain Technology, 38:231-235.

Tonna E.A., 1971. Topographical labelling technique used in assessment of skeletal growth, remodelling and development. Journal of Dental Research, 50, 1507 (abstract).

Tonna E.A., 1974. Topographical labelling method using ^{3}H-proline in assessment of skeletal growth and remodelling in 5-week-old mice. Laboratory Investigation, 30, 161-169.

Tonna E.A., 1975. Application of the H^{3}-proline topographic method to dental tissues. Journal of Periodontal Research, 10, 357-368.

Tonna E.A., 1976. Topographic labelling method using {^{3}H}-proline autoradiography in assessment of aging, parodontal bone in the mouse. Archives of oral Biology, 21, 729-774.

Trott J.R., 1962. The development of the periodontal attachment in the rat. Acta Anatomica, 51, 313-328.

Waugh I.L.M., 1904. The alveolodental membrane: its minute structure from a practical standpoint. Dental Cosmos, 46, 744-747.

Zwarych P.D. and Quigley M.B., 1965. The intermediate plexus of the periodontal ligament: history and further observations. Journal of Dental Research, 44, 383-391.