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Review Article

Periodontal and Dental Follicle Collagen in Tooth Eruption

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Abstract

Review of the process and mechanism of tooth eruption defines active eruption as coronal migration of the tooth to the functional occlusal position in the oral cavity while passive eruption occurs by loss of epithelial attachment to expose the clinical crown. Rodent teeth are considered excellent analogs of eruption because they have examples of limited and continuous eruption in the molar teeth and incisors respectively. Root resection studies on rat incisors exhibit normal active eruption rates due to a 'force' in the retained periodontal ligament (PDL). Since all four walls of the tooth and bone remain patent it confirms that the periodontium alone is the prime mover of eruption. Impeded and unimpeded eruption rates were grossly retarded when a collagen crosslinking inhibitor lathyrogen 0.3% AAN (aminoacetonitrile) was added to the drinking water of young 45 - 50 gm rats. Using the Bryer 1957 method of measurement of eruption it appeared that low concentrations (0.01%) lathyrogen in the drinking water of adult rats did not have significant retardation of unimpeded eruption rates. Histological, radiological, bone and tooth marker studies indicate that intrusion and dilaceration of the reference molar and incisor decreases impeded eruption in the lathyritic condition giving a false impression of increased unimpeded eruption. Fixed metallic reference in the alveolar bone shows that even low dose of lathyrogen retards impeded and unimpeded eruption rates. It is now clear from high voltage and conventional transmission electron microscopy that collagen assembly in vivo is a linear and lateral aggregation with crosslinking of collagen segments occurring in a variety of species which increases isometric contraction/stiffening of the extracellular matrix. Given that lathyrogen reduces intramolecular and intermolecular crosslinks it is concluded that collagen maturation plays a prime role in tooth support and eruption. The physical state of the strain stiffening of aligned isometric collagenous substrate is essential to cell differentiation in the cell-extracellular matrix contraction. Thus, this study of eruption demonstrates that its an excellent study for cell differentiation and migration.

Keywords: *Periodontal and Dental Follicle; Collagen; Tooth Eruption*

Introduction

Prior to 1960 tooth eruption had been considered a complex question primarily because it was believed that pressure forces alone 'pushed' the developing tooth out of the bone to and through the oral cavity to contact the opposing teeth in the occlusal plane. In subsequent years it has become progressively clear from research investigations that tooth eruption is due to tractional force in the extracellular cell-collagen matrix (ECM) of the dental follicle and periodontal ligament [1-9].

Berkovitz BKB and Thomas NR [10] demonstrated that transected rat teeth continue to erupt at control levels from 4 days after removal of the proliferative odontogenic base retaining that rate for 12 days until the base of the tooth approached the level of the alveolar crest. None of the traditional pressure factors of tooth eruption 1) root elongation 2) pulp cell proliferation and dentine formation 3) fundic bone deposition, 4) tissue fluid pressure 5) cushioned hammock tissue, are essential to the force of tooth eruption. Interstitial fluid pressure as a traction force depends upon its location in an enclosed system within walls.

But all walls were removed in the root resection surgery and remained patent throughout the experiment and cannot therefore be a source of prime mover in tooth eruption. Especially is this so given that the widest diameter of the apex of root chamber of the incisor of rats is at the root apex and any distal pressure would serve to push the tooth apically i.e. back into the socket defined as intrusion. Tissue pressure forces did not appear to be likely contenders as eruptive forces but rather as resistance forces acting against the prime mover of eruption in the PDL. Thus, blood pressure theories are also dismissed as an eruptive force per se. Pulsatile blood flow could act as a trigger to the mechanical properties of the ECM under different levels of stress and varying solvent conditions measured by full-atomistic force field. Hydrated collagen microfibrils yield a modulus of elasticity of 300MPa at small and 1.2 GPa at larger deformations in excess of 10% strain in drier collagen fibrils exerting a Young's modulus of 1.8 to 2.25 GPa due to a tighter molecular packing of the microfibrils [11,12]. Thomas [13] had previously proposed such a force during intra and intermolecular collagen crosslinkage in the PDL and dental follicle. Under regulation of lysyl oxidase and connective tissue growth factor by TGF-beta this sequential process is reviewed by Hong., *et al*. [14] and Trackman [15].

In particular the question emerges why the histological character of the periodontal fibroblast assumes a drawn-out spindle and stellate form. It was hypothesized [16,17] that the orientation of the fibroblast was related to alterations in the cell surface arising from the polymerization of collagen fibrils as they are secreted alongside the cell membrane into the ECM. The evidence provided by these and other studies indicate that tropocollagen molecules form in close association with the fibroblast cell surface and act as the push/pull or slip of the prime formative units of collagen and shown to have an adhesive strength that is highly sensitive to the pulling rate and that converges to a value of 10.12 pN/A for vanishing loading rates which given the thickness of a rat PDL at about 0.1 mm is of the order of 1.0N across the ligament acting between collagen microfibrils while its consequent stiffening produces an isometric tenacity stretching coronally from tooth cementum to alveolar bone along an oblique structured ECM. Gautieri., *et al*. had reported the analysis of the ECM's elastic properties under different levels of stress in varying solvent conditions. Using a full-atomistic force field including explicit water solvency of hydrated collagen microfibrils yields a Young's modulus of 300MPa at small deformations. Dehydrated dry collagen microfibrils show a significantly increased Young's modulus of 1.8 to 2.25 GPa or 6.75 times the modulus in the wet state owing to a much tighter molecular packing of the microfibrils resulting in an axial force. Thus, the isometric traction property is engendered by and within the ECM. In response the fibroblast cell develops isotonic contraction essential for cell spreading, migration, and cell-matrix contraction by promoting intracellular strain defined cell 'prestress' and cell tensegrity. It is important to understand that the stiffness of the collagen substratum of the ECM is the source of cell tensegrity.

The slip of two micro fibril/ tropocollagen molecules (Figure 1) is due to the intermolecular H bonds which play a key role in determining the resistance against slip crucial to constitutive tissues at larger hierarchical levels [12]. In the latter study collagen nano fibrils are observed at the cell surface where they are secreted as procollagen and from which the non-helical end chain telopeptides are removed by peptidases [15] to form tropocollagen (TC) fibrils that serially line up in quarter staggered arrangement with volume space for water bridging. The collagen triple helix is a unique protein motif defined by the supercoiling of three polypeptide chains in a polyproline II conformation. It is a major domain of all collagen proteins and in several membrane proteins. The triple-helical domain has distinctive properties. Collagen requires a high proportion of the post-translationally modified imino acid 4-hydroxyproline to which water is added to stabilize its conformation and assembly. The crystal structure of a collagen-like peptide determined to 1.85 angstroms showed that these two features are related [18].

When the latter microfibrils form in bundles it appears as though templates coincide with resultant stress and strain within the cell cortex from the polymerization of fibril segments at the cell surface. It had been noted in these and earlier preparations that the fibrillar bundles passing over a cell surface are in parallel array and evenly spaced described as quarter staggered overlap by Hodge AJ and Schmitt FO [19] (Figure 2 and 3). The length of the procollagen monomer measures 3000 Angstroms but during polymeriza-

The triple helix structure of collagen a. Component chain wound in a left hand helix around a straight imaginary axis .The circles represent amino acid residues b. the same chain wound in a right hand helix c The axes of the three component chains to form a super helix, the polypeptide is omitted for clarity.

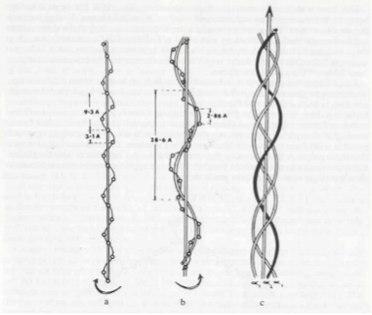


Figure 1

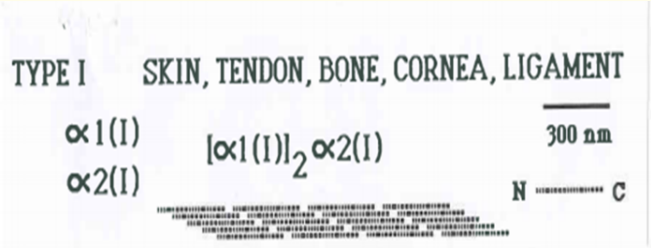


Figure 2

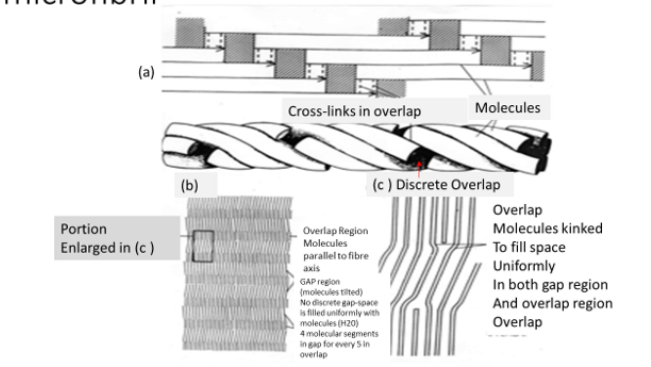


Figure 3

tion the telo peptides of the non-helical end chains are cleaved by peptidase to a length of 2800 Angstroms prior to joining by spontaneous polymerization in a quarter staggered arrangement giving a native collagen banding of 680 Angstroms indicating a shortening of each collagen monomer which leaves gaps and kinks known as collagen pleating or crimping which under tension give rise to the early 'toe' of stress/strain dynamic curve of young collagen stretching and flexibility probably due to a preferential give in the crosslinks and the changes in water content in lateral packing of the tropocollagen [20]. The collagen triple helix is a unique protein motif defined by the supercoiling of three chains in a polyproline II conformation. Collagen requires a high proportion of the post-translationally modified imino acid 4-hydroxyproline and water to stabilize its conformation and assembly. The crystal structure of a collagen-like peptide determined to 1.85 angstroms showed that these two features may be related. Detailed analysis of the hydration structure of collagen is in a semi-clathrate structure that surrounds and interconnects triple helices and no doubt contribute to tensile properties and play a crucial role in the assembly of collagen acting as interstitial and bridging waters. The hydroxyproline acts as a lynch pin [21].

These conformational properties of water bridging phenomena appear to have been misunderstood by Tyler and Burn-Murdoch (1975) who showed that incisors erupted coronally when deionized water was added to the PDL conforming to Bella., *et al*. [18] explanation that water bridging stabilizes the PDL collagen. Thomas and Tyler 1972 quoted in Thomas [13] also observed that physiological saline with glutaraldehyde (0.04M) restituted the cell -collagen matrix contractile properties of rat PDL and rat tail tendon. Of course, a 'fixing' glutaraldehyde concentration (0.5%) in the Tyler and Burn Murdoch investigation would clearly stop eruption since cell function is essential to the ECM contraction theory.

It is noteworthy that in many instances the direction adopted by collagen fibrils is normal to an axis of polarity of the fibroblast which passes through the centrosome and nucleus of the cell. It would seem that wherever the cells are freed from the stresses and distortions of migration they slow down and form a loose sheet, and that under these circumstances the fiber bundles which form, bear a definite relation to axes of the cells. It is difficult to imagine

how such cell to collagen relationship could be the result of anything other than an intimate association of matrix substrate and cell. Birk., *et al*. [22] demonstrated that collagen fibril formation *in vivo* is a multi-step process. Using high voltage transmission electron microscopy, they describe the complexity of three secretory channels as they become the cell surface. The first line channels have their origin within the enveloping cytoplasm approximating the Golgi region opening to the extracellular space while extending from deep within the cell. The second channel system fuse laterally within which collagen bundles form. Within this channel fibril segments coalesce to form discontinuous bundles opening distally into the extracellular matrix. In the third channel the bundle forming compartments continue to aggregate laterally the intervening projections of cytoplasm retracting and a compartment is generated within which broader bundles are generated. Thus, in this final fourth channel the collagen fibrils collect into larger bundles where fibrils join laterally and linearly by segment linking so that longer fibrils with larger diameters form in proximity and close association with the cell surfaces of two or three other cells. There linear and lateral packing occurs along the cell surfaces where the propeptides are removed by specific amino and carboxy proteinases and where fibril assemblies may be mediated by lysyl oxidase that catalyzes the final known enzymatic step required for crosslinking of collagen regulated by TGF-beta 1.

Bailey [23], quoted in Thomas (1975), argued that while 'collagen must play some role in tooth eruption' he questioned that collagen molecules within a fibre could, by contraction of the crosslink and/or telopeptide and the sliding of one molecule across another act in unison and simultaneously retain the same precise pattern of the native fibre. In fact I agree with Bailey regarding what may not happen for fibres but it is at the fibril stage that is significant to contraction and eruption. This is in effect what Birk., *et al*. (1990) observes and describes of the actual occurrence of fibril formation when examined under electron microscopy and computer assisted image reconstruction.

Fibril segments were found in extra cytoplasmic channels and in fibril bundles in a manner suggesting that the fibril segment is formed in the channel and then added to a bundle containing other segments of varying lengths. Electron microscope autoradiography demonstration indicates that fibril segments were newly synthesized and assembled. The lengths and diameters of the fibril segments and bundles were determined in serial sections. The ends of fibril segments were recognizable by their significantly smaller diameters and by their disappearance in the serial section set. A single fibril segment was followed from its beginning to its termination in consecutive segments. This fibril segment is approximately 8 ums in length. Other fibril segments were followed and the lengths varied from 7 to 15 um. An asymmetric fibril segment would be consistent with work on fibril growth *in vitro* where the amino-terminal end of the growing collagen fibril was found to decrease by 4 to 5 molecules per 67 nm. While the change at the carboxyl end terminal end was 8 to 10 per 67nm. That description fits what I had hypothesized at the Colston Symposium in the statement: 'The interaction of adjacent double layers is the source of a strong repulsion between the collagen molecules. A predominantly axial orientation of the collagen fibrils leads to repulsive forces which are strongest in the direction normal to the fibre axis. The repulsive field (Debye length-the ionic layer of the solution around the fibrils) becomes:

$1/k=(ekt/ECZ_2e_2)^{1/2}$ where C is concentration of the i^{th} species e is the electronic charge, T temperature and Z is the valence. This interfibrillar repulsion gives rise to a substantial axial component of force when the fiber is constrained at either end. Under such a strain the collagen molecules and fibrils may be prevented from sliding over each other due to the intermolecular crosslinks between the tropocollagen crosslinks. Certainly, it is anticipated there would be a push and pull on the fibrils as they polymerize in the soluble phase before entering the insoluble phase when just two nano fibrils develop Newtonian force across the PDL as described above by Gautieri., *et al* (2009). It is becoming increasingly apparent with advancing research that polymerization and crosslinking of collagen occurs close together while increasing the isometric tension and stiffness of the extracellular matrix which warrants the definition given it by cell physiologists as 'collagen matrix contraction' and tension. Figure 3 shows the site of crosslinkage approximate to the gaps which fill with water in the young periodontium and rat tail tendon to produce the increased 'toe region' of the young stress-strain curve [20].

Certainly, it explains how lathyrogen in drinking water could result in retarded eruption (see below). Without direct proof the work of Ingber [24] and Wang., *et al*. [25], Mih., *et al*. [26] and Huang [27] describe 'cell tensegrity' as the tension on the cell surface that

leads to cell 'prestress' in which the cell triggers the formation of actinomyosin causing the stem cell to proliferate and form contractile cells as they differentiate into myofibroblasts as also discussed by Thomas [28].

Trelstad [29] provides diagrams of 12 types of collagen which include tissues of the PDL depicted in figure 1 and 2 which is the Hodge and Schmitt quarter staggered structure and alignment of Types 1, II, III, V and XI which are also found in the tissues of the PDL. Fleischmajer., *et al*. [30] states that Type 1 and type III collagens are interstitial collagens that have the property to polymerize in the extracellular matrix and form fibrils with a characteristic 67 nm periodicity. Recent data suggest that various fibril forming collagens may coexist within individual fibrils and may particularly be the case for Types I, II, III, V and XI. Contrary to the dental literature it seemed apparent that PDL collagen and cell were influencing each other. Thus, one could reach the tentative conclusion that tooth eruption was rather due to the development of tractional force in the dental follicle and periodontal tissues which 'pull' the tooth through the tissues to meet the opposing teeth in the occlusal plane. Birk., *et al*. describes the formation of fibril segments in the extra cytoplasmic compartments in 14-day chick embryos and found it consistent in a variety of other species. The assembling fibrils increase laterally to a full diameter fibril with a bimodal distribution of 1.6 and 4.5 microns corresponding to pointed endings at the N end and round ends at the C ends of the collagen peptides. By movie BYU they concluded that at least 60 to 80% of the fibrils are present as fibril segments and are staggered with respect to one another and in highly anisotropic alignment. They predict that there is lateral and/or linear fusion without loss of the 67 nm banding. The fusion of the fibril segments probably occurs via a surface coat of dermatan sulfate proteoglycan. In summary it is apparent that the findings are in agreement with those presented at the Colston Symposium 1975 where Bailey (1975) argued that linear fusion of collagen fibres is unlikely to occur because the 67 nm banding of collagen would be lost. While this is firmly denied with convincing findings for fibrils by Trelstad (1990) the pronouncements of Bailey have spurred research and we are grateful to him although it was, at the time, hard to adapt. Fibroblast-collagen-matrix contraction occurs at the surface of the fibroblast where cell membrane ruffling by PDGF platelet growth factor and lysophosphatidic acid stimulation component of cell Rac and Rho actions of cell migration uncouples from myosin light chain phosphorylation which informs that while actinomyosin cell contraction is one component of cell migration it is NOT the complete answer [31-33] regarding components of cell migration across ECM.

Materials and Methods

Tetracycline marker studies

For the purpose of this study 50 rats were given multiple intra peritoneal injections of a normal saline solution containing 10 mg/ml of tetracycline from birth to 3 days (8 rats) sacrificed at 3 and 7 days; on 3 to 44 days (8 rats) sacrificed at 44 days; on 5 and 44 days (9 rats) sacrificed on 46 day; 7 days (3 rats), sacrificed on 12 and fourteen days; on 1, 7, 16, 21 and 30 days (6 rats) sacrificed 7, 16, 21 and 30 days; on 16, 22, 32 and 36 days (9 rats) sacrificed on 16, 22, 36, 45 days; on 16, 22, 36, 45 days (7 rats) sacrificed on 16, 22, 36, 45 days at a dose of 20 mg/Kg from birth to 40 days Rats were fed a normal pellet diet with water provided ad lib. The measurements were made along the vertical axis of the mesial cusp of the first mandibular molar tooth. In order to arrive at the correct plane of section a stereotactic method of cephalostat preparation was designed to maintain accuracy and comparability of sections. In the embedding procedure commercial methacrylate monomer was used and the jaw specimens were processed in an incubator at 37 degrees centigrade and suspended in an articulated blocking tank which is filled with monomer and accelerator. The sections were made along the vertical axis of the developing tooth. The whole specimen and the sections were examined and photographed by fluorescent light microscopy (Figure 5 and 6).

Figure 4

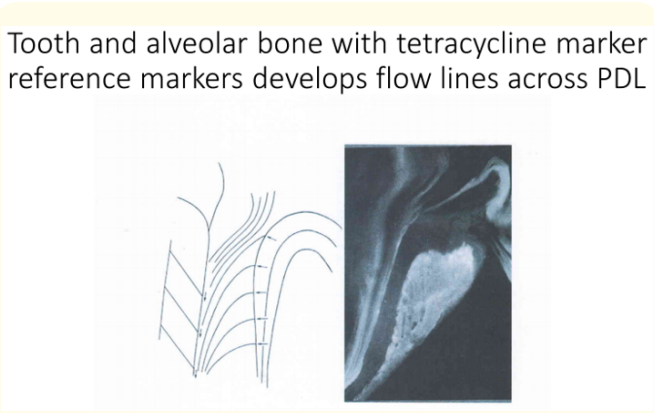


Figure 5

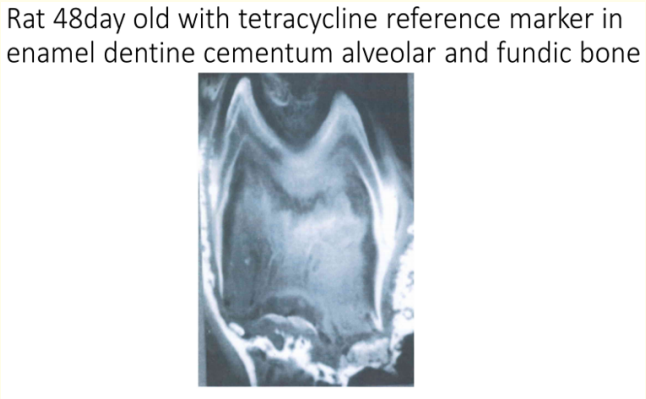


Figure 6

Radiography

In order to record skull and mandibular growth of normal and later lathyritic rats a simple cephalostat was designed for lateral radiography. The methacrylate half block containing skull and mandible is radiographed. The film was placed in an enlarger and tracings were made on fine tracing paper. The variation in readings did not exceed 1 per cent. The osseous landmarks employed in figure 7 are the cephalometric radiological determinants of normal molar and incisor eruption viz: upper diastema (A-B), lower diastema (C-D), distance between occlusal surface of first lower molar and superior aspect of lingual bone (E-F), extra-alveolar portion of mandibular incisor (C-G) and extra-alveolar portion of maxillary incisor (A-E), height of anatomic crown of first mandibular molar (E-N), length of anatomical root (N-P), the relative position of cemento-enamel junction to the alveolar crest (N-D), the distance between the apex of the mesial root and the lingual surface (P-R) of the incisors.

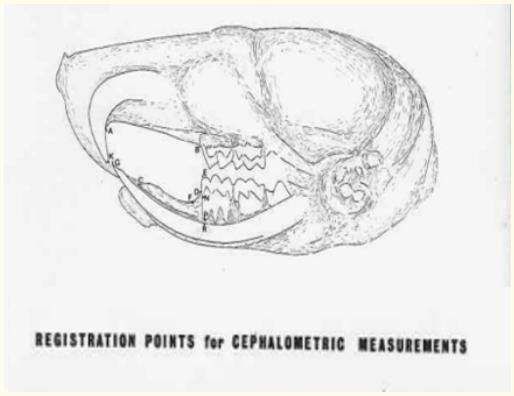


Figure 7

Tooth growth and first stage of eruption as well as supporting bone measurements using tetracycline markers are shown in figure 8. It is observed that molar root first grows down towards the inferior alveolar canal by 88 microns until on the twelfth day eruption commences reaching almost a millimeter in the following first week. Thus, there is no follicular eruption only growth of the tooth germ of a five-day rat with resultant bone resorption above, below and around the dental follicle (Figure 9). It will be seen that as the root forms it begins to erupt with evidence of fundic bone formation in figure 10 then the center of the follicle does bodily migrate coronally as summarized in figure 11 end diagram. At this stage collagenous microfibrils thicken as seen in the tooth histology (Figure 12) of the follicle and in the PDL of the twelve-day molar (Figure 10).

Process of eruption

Measurements of normal eruption

In figure 4 we observed the tetracycline markers in the normal developing mandible. It will be noted that under ultraviolet light there is a fluorescence of the alveolar bone, the incisors and molar teeth. The body and lower border of the mandible shows no fluorescence indicating that the eruptive process was preceded by formation of the basal bone of the jaws except for the condyle which is a secondary growth center which ossifies by endochondral ossifi-

Normal Rat First Molar Growth and Eruption from Tetracycline Marker Studies

Normal Rat First Molar Growth and Eruption Measured From Tetracycline Marker Studies

Age (days)	Root growth microns	Fundic bone growth microns (initial resorption 88 microns)	Alveolar bone growth microns	Eruption (mean of three methods*) microns
12-19	954	54	203	880
19-26	640	51	163	701
26-33	402	45	166	450
33-40	108	48	168	154

*Methods of measuring eruption: 1. Alveolar crest tetracycline marker to cementoenamel junction. 2. Fundic bone plus root growth. 3. Interradicular bone growth from day 14.

Figure 8

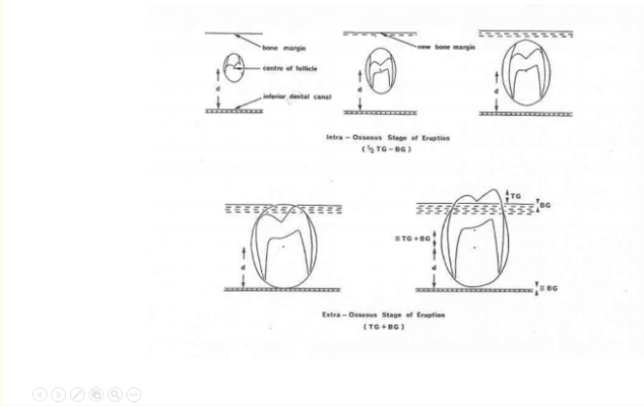


Figure 11

5 day old rat. Note resorption of fundic bone beneath the developing molar and incisor

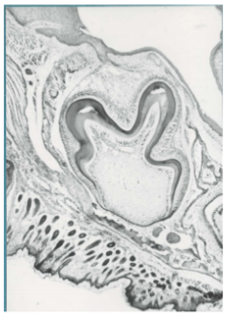
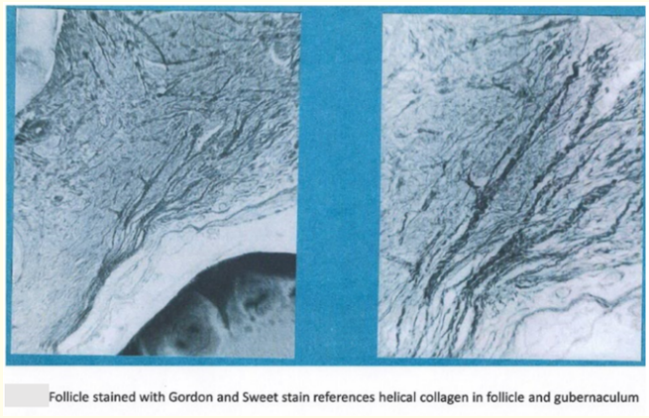


Figure 9



Follicle stained with Gordon and Sweet stain references helical collagen in follicle and gubernaculum

Figure 12

Histology of teeth in situ prior to lathyrism

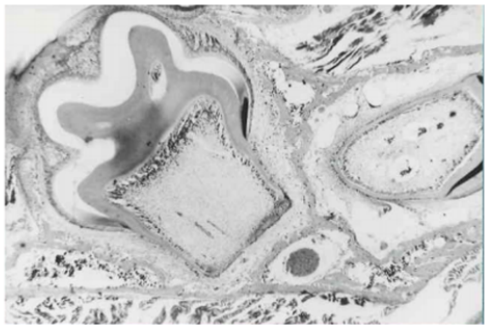


Figure 10

cation. It thus serves to keep the height and length of the mandible consonant with the growth and eruption of the teeth. From this we conclude that the eruption of the teeth requires increase in the intra oral space achieved by an increase in vertical dimension of the jaws accommodated by growth of the condyle to increase the rise of the occlusal plane. Thus, we were able to confirm by histology that the inferior alveolar nerve once formed beneath the forming mandibular teeth is relatively fixed and can be used to measure all phases of eruption especially during the follicular and periodontal stages of tooth development.

Figure 9 presents a section through a first mandibular molar of a rat at five days after birth. It is observed that the molar continues to grow from a fixed center in the fossa. It will be noted that the molar is at the follicular stage of intraosseous development where there is little or no eruption.

In figure 10 is a developing dental germ and follicle with the dental canal below. The center of the tooth germ allows the measurement of eruption if any as: ½ TG (tooth growth)-BG (Bone growth (above the tooth). The center of the dental follicle is fixed in relation to the nerve canal while the tooth simply grows in the follicle. Eruption in the rat does not occur until after the root forms on the twelfth day. It is acknowledged that the PDL is formed from downgrowth of the dental follicle tissue.

Figure 13 provides a table of measurements made from the radiological data of eruption of the first molar amelocemental junction to the lingual surface of the rat incisor. The results are represented graphically in figure 14. There is no eruption until the fourteenth day despite the fact that the root growth commenced on the eleventh day when it is seen from the marker studies that there is resorption of the fundic bone. There is therefore a latent period of three days of tooth growth before the eruptive force reveals itself. Supporting evidence of this was available from alizarin injected animals. In these three days the new root growth is covered by cementum during which time collagen of the PDL is incorporated as Sharpey's fibres. The tooth during this time is subjected to blood pulsations which result in developing the stress strain elastic modulus of the PDL collagen being formed and crosslinked which Thomas 6a, b 1967 demonstrated in radioactivity of tritiated glycine in ECM incorporated into every three amino residues in every turn of the collagen helix turnover every 8 days is reinforced by crosslinkage. The newly formed collagen is at first soluble that becomes progressively insolvent during which time crosslinking occurs as the proteases remove the telopeptides to form the TC molecular segments.

Based upon the hypothesis of collagen as a generator of tooth eruption it was decided to set about observing the effect of preventing the maturation of collagen in the periodontium of erupting teeth by adding lathyrogen known to inhibit collagen 'stiffening' by adding varying concentrations of Aminoacetonitrile bisulphate and Beta propionitrile to the drinking water of rats. Collagen cross-

ERUPTION of NORMAL FIRST MOLAR OF RAT

Days	Height of crown in mm	Length of root in mm	Eruption (CEJ) in mm	Fundic bone in mm	Alveolar crest in mm
7	1.2	0.0	0.0	-0.10	1.25
12	1.3	0.24	0.04	-0.30	1.47
14	1.3	0.36	0.45	0.09	1.55
21	1.27	1.13	1.30	0.14	1.85
25	1.26	1.35	1.53	0.18	2.05
35	1.20	1.62	1.84	0.23	2.15
42	1.14	1.80	2.05	0.27	2.25
49	1.10	1.91	2.04	0.32	2.30
56	1.05	2.01	2.40	0.36	2.34
63	1.01	2.10	2.51	0.40	2.40
70	0.95	2.16	2.60	0.43	2.45
77	0.92	2.20	2.68	0.46	2.50
84	0.90	2.25	2.73	0.48	2.52
91	0.87	2.31	2.83	0.52	2.55
98	0.85	2.34	2.91	0.54	2.60
105	0.85	2.40	2.99	0.58	2.62
112	0.85	2.43	3.05	0.61	2.65
119	0.84	2.46	3.09	0.63	2.68
126	0.84	2.49	3.14	0.66	2.72
133	0.84	2.51	3.17	0.69	2.75
140	0.84	2.53	3.23	0.71	2.77

Figure 13

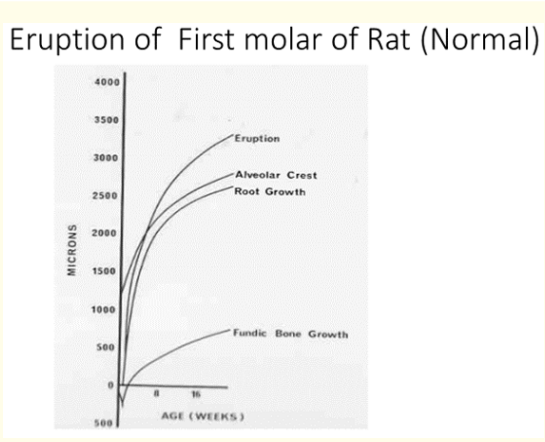


Figure 14

linkage converts tropocollagen monomer fibrils from a soluble fluid like state to insoluble tensile collagen fibers prevented by lathyrogen known to inhibit crosslinking of collagen by which tropocollagen alongside other tropocollagen monomers opposes applied forces through the action of its intramolecular hydrogen (H) bonds. Thus, lateral packing of tropocollagen molecules and hence water loss from the spaces between the molecules provides a substantial force of eruption as demonstrated in the stress strain of the toe of young collagen stretching forces exerted for example by migrating periodontal cells.

Results of the effect of lathyrogens

Eruption of young rats utilizing 0.1% AAN (aminoacetonitrile) in the drinking water it was found that the eruption of the molar

and incisor are retarded (Figure 15 and 16) with resultant downgrowth of the roots to resorb the fundic bone to a minimum despite the continuing growth of the teeth which are dilacerated. Nevertheless, in figure 17 the measurements clearly show that the maxillary and mandibular diastemata are decreased by 500 to 750 microns and 200 - 500 microns in 21 days respectively, impeded eruption of mandibular molars by 750 microns and 300 - 600 microns and 200 - 700 microns for incisor eruption for a low dose of lathyrogen in the drinking water when compared with pair fed controls. The molar and incisor roots show gross dilaceration (Figure 21) so the occlusal plane is also necessarily decreased. In figure 19 and 20 it is observed that tooth eruption of incisor teeth in and out of occlusion i.e. impeded (in occlusion) and unimpeded (out of occlusion by cutting off the crown to the gingival margin) respectfully show marked retardation of eruption rates of impeded and unimpeded young rat incisors when administered a greater dose of lathyrogen, acetoaminonitrile (AAN) at 0.3% in the drinking water.

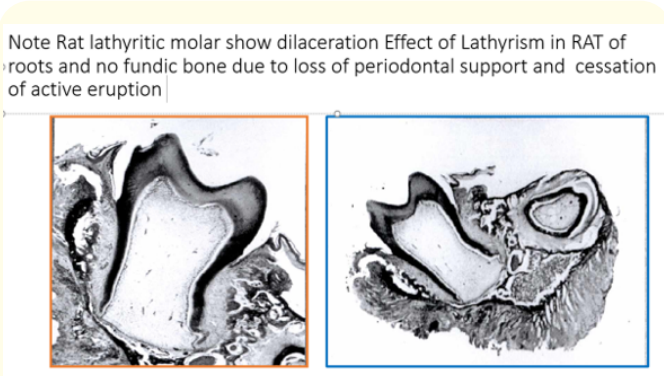


Figure 15

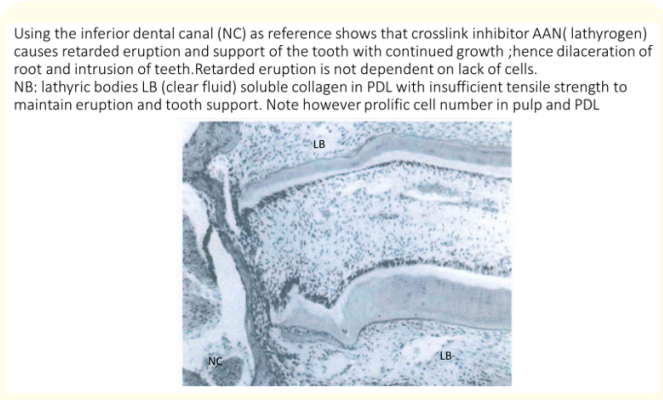
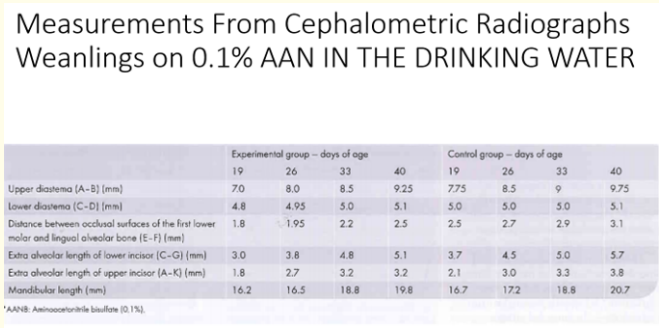


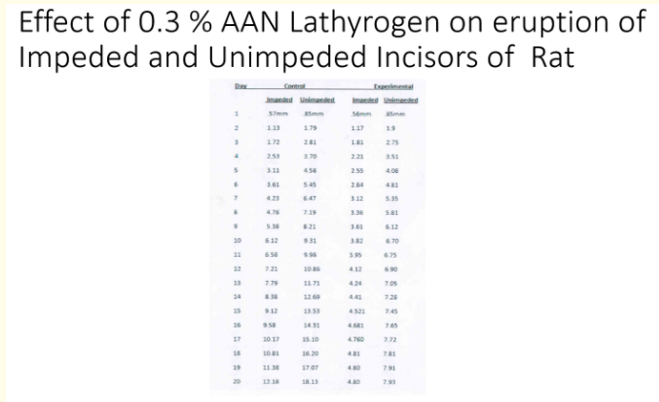
Figure 16



	Experimental group – days of age				Control group – days of age			
	19	26	33	40	19	26	33	40
Upper diastema (A–B) (mm)	7.0	8.0	8.5	9.25	7.75	8.5	9	9.75
Lower diastema (C–D) (mm)	4.8	4.95	5.0	5.1	5.0	5.0	5.0	5.1
Distance between occlusal surfaces of the first lower molar and lingual alveolar bone (E–F) (mm)	1.8	1.95	2.2	2.5	2.5	2.7	2.9	3.1
Extra alveolar length of lower incisor (C–G) (mm)	3.0	3.8	4.8	5.1	3.7	4.5	5.0	5.7
Extra alveolar length of upper incisor (A–K) (mm)	1.8	2.7	3.2	3.2	2.1	3.0	3.3	3.8
Mandibular length (mm)	16.2	16.5	18.8	19.8	16.7	17.2	18.8	20.7

*AANB: Aminoacetonitrile bisulfate (0.1%).

Figure 17



Day	Control		Experimental	
	Impeded	Unimpeded	Impeded	Unimpeded
1	.57mm	.85mm	.56mm	.85mm
2	1.13	1.79	1.17	1.9
3	1.72	2.81	1.81	2.75
4	2.53	3.70	2.21	3.51
5	3.11	4.58	2.55	4.08
6	3.61	5.45	2.84	4.81
7	4.23	6.47	3.12	5.35
8	4.78	7.19	3.36	5.81
9	5.38	8.21	3.61	6.12
10	6.12	9.31	3.82	6.70
11	6.58	9.96	3.95	6.75
12	7.21	10.86	4.12	6.90
13	7.79	11.71	4.24	7.05
14	8.38	12.69	4.41	7.28
15	9.12	13.53	4.521	7.45
16	9.58	14.31	4.681	7.65
17	10.17	15.10	4.760	7.72
18	10.81	16.20	4.81	7.81
19	11.38	17.07	4.80	7.91
20	12.16	18.13	4.80	7.93

Figure 18

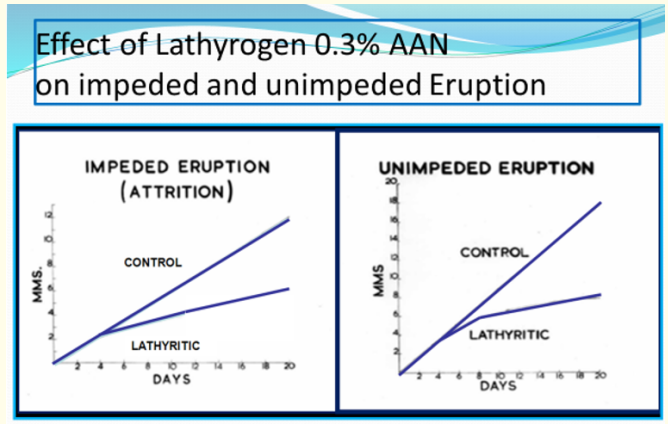


Figure 19

Eruption Rates of Incisors Control and Lathyritic (Beta amino propionyl nitrile BAPN)

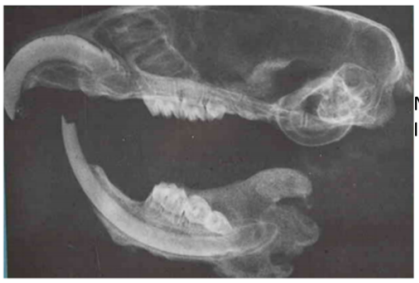
Eruption rate	Using occlusal plane (mm)	Amalgam Pellet (mm)	Difference
Lathyritic - unimpeded	16.24 (SD=1.19)	13.75 (SD=0.635)	2.49
Control - unimpeded	17.01 (SD = 1.13)	15.37 (SD=0.38)	1.64
Difference	0.77 (4.5%) +	1.62 (10.4%) *	
Lathyritic - impeded	10.57 (SD=1.4)	8.08 (SD=0.08)	2.49
Control - impeded	11.42 (SD=1.2)	9.78 (SD=0.76)	1.64
Difference	.85 (7.4%) +	1.7 (17%) *	

* Statistically Significant p < 0.001
+ Not Statistically Significant p > 0.05

The difference between results using the occlusal plane and amalgam pellets is explained as underestimation in the former due to the intrusion of the impeded teeth.

Figure 20

Lathyritic Cranium and Mandible



NOTE DILACERATED INCISOR IMPEDED ROOTS

Figure 21

Berkovitz., *et al*. [34] team undertook a similar investigation on 90, 180 and 300g rats using 0.1 AAN in the drinking water. The youngest group showed significantly lower eruption rates which supports our radiography findings in this study. However, in Berkovitz., *et al*. (Figure 22) there is retardation of eruption for 8 days. But from the 12th day the impeded teeth appears to show no significant difference due to intrusion, root fracture, dilaceration of the impeded tooth and loss of fundic (basal) bone which as the reference tooth erroneously exaggerate the eruption of the unimpeded tooth when using the faulty measurement method of Bryer [35] which has obscured in all previous findings on eruption in the dental literature including Chiba., *et al*. [36] and Tsurata., *et al*. [37] who simply micro photographed the erroneous eruption measurements not observing the tooth intrusions, gingival recession nor altered occlusal plane while conceding inflammation of the reference gingiva as seen in this study and exemplified by figure 3-7 (see also Thomas 2020 [28] for further details) featuring the bending, dilaceration, fracture and intruded reference teeth which has led to widespread miscalculation of eruption and mislead countless dental researchers in the scientific literature to date since none have undertaken the critical study of the present work of using a fixed metallic reference which is essential. But six years later Michaeli., *et al*. [38] understood the effect of tooth intrusion and repeated the study by cutting the impeded tooth once out of occlusion and resolutely confirming Thomas 1966.

Lathyritic (AAN 0.1%): Large Rats 180-300gm Compared with controls over 24 Days Berkovitz et al 1972

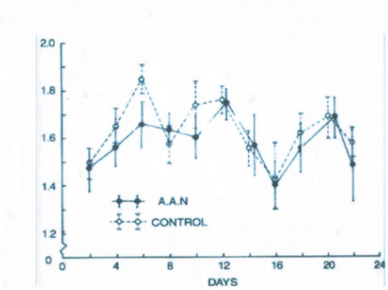


Figure 22

Conclusion

The resected rat incisors exhibit normal impeded (occluding) and unimpeded (non-occluding) active eruption. It is evident that the force of eruption resides in the retained periodontal ligament (PDL). The PDL retains its characteristic histological structure following root resection. The continued normal eruption appears to be due to a tractional force within the PDL. Tropocollagen (TC) monomers are originally 3000 angstroms in length known to line up and polymerize consequent to losing their non-helical end chains to become 2800 angstroms in length. The latter polymerizing in a quarter stagger alignment with water retained in the collagen gaps where crosslinking also takes place and shows the concealed bending of the overlap regions seen in figure 3 gap spaces between the TC molecules that laterally and linearly pack thereby squeezing water out of the polymer without losing the banding at 640 angstroms which 'stiffens' into the formed collagen fibrils engaging in crosslinking. Lathyrogen known to prevent crosslinking is given to the rats in the drinking water. Measurements using

tetracycline reference markers, cephalometric radiology using relevantly fixed anatomical points including the Inferior Dental Canal and amalgam reference markers in the alveolar bone alongside impeded incisor and molar teeth (in occlusion) and unimpeded incisors where the crowns are removed at the gingival margin and hence not in occlusion [39]. Both impeded and unimpeded eruption rates are grossly retarded when collagen crosslinkage is inhibited by lathyrogen supporting the hypothesis of eruption as a collagen matrix tractional force including the observation that the PDL collagen polymerization and stiffening is a supporting mechanism of the tooth against occlusal forces and attrition.

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