Histomorphological Study of Dentine Pulp Complex of Continuously Growing Teeth in the Rabbits

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Abstract: Background: Rabbits have a diphyodont dentition (permanent and deciduous sets of teeth). Rabbit teeth are also heterodont (of different types)**.** Incisors as well as posterior cheek teeth are *aradicular hypsodont*, indicating that the teeth have a long anatomic crown and do not have true roots. These teeth erupt continuously, and remain open-rooted. **Aim:** The present study aims to clarify the morphological and histological features of the dentine pulp complex of continuously growing incisors & molars teeth of rabbits. **Methods:** Ten male New Zealand white rabbits in the age of three months old were used in this study. After scarification, the lower jaws were dissected out and hemisected in sagittal direction into two halves (right and left sides of the mandible), Then prepared for Morphological examination using sterio microscopy and histological examination of decalcified and ground sections using light microscopy. **Results:** sterio microscopic examination revealed that molar teeth consist of two laminae with double apical openings. Histological examination for the rabbit teeth showed dentine precipitation along the side of the pulp and complete obliteration of the coronal portion of the pulp by osteodentine. **Conclusion:** different types of dentine are precipitated within the pulp chamber of continuously growing teeth as a compensatory mechanism for continuous teeth wear out.
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Keywords: rabbit cheek molar teeth; dentine pulp complex; osteodentine.

1. Introduction

Rabbits are small mammals fall into the order Lagomorpha (Lagomorpha means 'hare-shaped'), **family** *Leporidae* , and **genus & species** *Oryctolagus Cuniculus* [(1)]. There are many species of rabbits. Rabbits are herbivores (plant eating) and have a high reproduction rate. The teeth of the rabbit have some characteristic mammalian features; they are heterodont and diphylodont [(2)]. Rabbits have a diphyodont dentition (i.e. two successive dentition, deciduous and permanent dentition). They usually shed their deciduous teeth before (or very shortly after) their birth, and are usually born with their permanent teeth [(3)]. However, Navarro *et al.*, [(4)] reported that the eruption of deciduous molars of the mandible begins at four days after birth and of the mandibular permanent molars at nine days, while that of mandibular premolars occurs at twenty three days, replacing the mandibular deciduous molars which have exfoliated. At thirty two days all the mandibular permanent cheek-teeth are erupted. Moreover, at birth the mandibular deciduous molars are completely developed and at four days their root resorption is initiated.

The teeth of rabbits complement their diet, which consist of a wide range of vegetation. Since many of the foods are abrasive enough to cause attrition, rabbit teeth grow continuously throughout life. All permanent teeth in rabbits are elodont (i.e., continuously growing, “open-rooted”) [(5)]. Elodont teeth never form anatomical roots, the exposed part of the tooth is referred to the "clinical crown" the embedded part being the "reserve crown", with a germinal, growing, region at the apex [(6)]. All teeth of the rabbit are aradicular hypsodont, which means that there is an anatomic long crown but no true root. The crown is divided into supra-gingival and sub-gingival parts. The latter part is also called the reserve crown [(7&8)]

The teeth of the rabbit are also heterodont. Heterodont teeth are simply teeth of different types as opposed to teeth of the same type, called homodont. Rabbits have incisor teeth and cheek teeth. The cheek teeth include both premolars and molars. Rabbits do not have canine teeth as in cats, dogs, ferrets and hedgehogs. The deciduous dentition (16) is as follows: maxillary: two incisors and three molars; mandibular: one incisor and two molars. The permanent teeth (28) are maxillary: two incisors, three premolars, and three molars; mandibular: one incisor, two premolars, and three molars. **Anatomic Dental Formula** 2(I 2/1 C0/0 M3/2) = 16 deciduous teeth and 2(I2/1 C0/0 P3/2 M3/3) = 28 permanent teeth. In the adult rabbit, upper two incisors are situated in each premaxilla; one is located behind the other. The posterior incisor is smaller than the anterior one. Its existence is a characteristic feature of the lagomorph family. There is only one incisor in the mandible [(2)]. Lagomorphs are distinguishable from rodents in that they have two pairs of upper incisors (the second pair, located immediately behind the larger incisors, are small and peg shaped, often referred to as the "peg teeth"); rodents have one pair [(9)].

Lagomorphs also have more teeth than rodents (especially, premolars). The incisors of both lagomorphs and rodents are "aradicular hypsodont" therefore, they continually grow throughout life. Lagomorphs and the truly herbivorous rodents (such as the chinchilla and guinea pig) feed on tough, fibrous vegetation in their natural environment; other rodents, such as hamsters, rats, and mice feed mainly on tubers (a fleshy, underground part of a plant, such as a potato), seeds, and grain. In the true herbivores, the diet tends to have a low energy content requiring the intake of larger quantities of food, resulting in more grinding of the vegetation and rapid wear to the cheek teeth (large teeth in the back of the mouth used for grinding). As a result, the cheek teeth, like the incisors, have evolved to continuously grow throughout life. In other rodents, the diet of tubers, seeds, and grain requires little chewing, resulting in little wear of the cheek teeth. As a result, these cheek teeth have anatomical roots and stop growing once they have fully erupted [(2&10)].

Rabbits have a typical herbivore occlusion: The premolars and molars are grouped as a functional unit with a relatively horizontal occlusal surface with transverse enamel folds (i.e., lophodont type of teeth: lophos ridge) for shredding and grinding tough fibrous food. The occlusal surfaces of these teeth consisted of three tissues of different hardness (enamel, dentine and cementum) arranged in different layers. The premolar teeth are similar in form to the molar teeth, and are usually described together as the 'cheek teeth'. They are closely apposed and form a single functional occlusal grinding surface. The teeth of species with abrasive diets resulting in rapid tooth wear. The most extreme modifications of the teeth generally occur in herbivores. The cheek teeth, premolars and molars, are modified with multiple folds of tooth structure, forming ridge patterns that help in reduce the rate of tooth wear [(11)].

Some authors use the term *aradicular hypsodont*, indicating that the teeth have a long anatomic crown and do not have true roots. These teeth erupt continuously, and remain open-rooted [(1)]. Open rooted refers solely to the large apical opening present in all continuously growing teeth [(12)]. Three to four millimeters of tooth is worn away by incisors every week, whereas the posterior teeth require a month to wear away the same amount [(13)].

In the normal rabbit, the eruption and growth of the incisors continues at a rate of 2-3 mm a week. The incisor growth and wear are balanced [(14)]. Therefore the diet must contain abrasive particles to ensure this equilibrium between growths and wear [(15)]. Mandibular incisors and cheek teeth grow and erupt faster than maxillary teeth [(16)].

The dental pulp is a highly specialized mesenchymal tissue characterized by the presence of odontoblasts and it is surrounded by a rigid mineralized tissue. The primary function of the dental pulp is to form dentine. Other functions include the nutrition of dentine and the innervations and defense of the tooth [(17)]. The dental pulp is infiltrated by a network of blood vessels and nerve bundles emanating from the apical region [(18)]. Dentin presents a tubular structure that maintains it in an intimate relationship with the pulp tissue through the odontoblastic process [(19)].

A large amount of research into the mechanisms of tooth growth and eruption has been performed using elodont incisor teeth, principally in guinea pigs, rats and mice, but little has been done undertaken regarding continuously erupting teeth of the rabbit incisor, premolars and molars. The present study aims to clarify the morphological and histological features of the dentine pulp complex of continuously growing teeth of the rabbits.

2. Material and Methods:

Ten male New Zealand white rabbits in the age of three months old were used in this study. After sacrification, the lower jaws were dissected out and hemisected in sagittal direction into two halves (right and left sides of the mandible). Then they prepared for the following examinations:

1- Morphological examination:

Some of the right sides of the mandible were stored in buffered solution (4.6 formaldehyde solution). Each side was embedded in a quick setting transparent blocks (bioplast). The bioplast consisted of base, catalyst and accelerator which were mixed properly just before use, then poured in suitable rectangular plastic containers. Initial setting of the mix was reached after about 8 hours, while complete setting occurred after 24 hours. After complete setting the blocks were removed from the plastic container and cutting sagitally into two half in mesiodistal direction and parallel to the axial plane by using Bronwill sectioning machine having diamond disc under water spray. Each half was kept without polishing. The sectioned surfaces were examined and photographed using the Stereo microscopy (Leica S 8Apo) to reveal the morphological characteristics of rabbit incisor& molar teeth.

2- Histological examination using decalcified sections:

The left sides were fixed immediately in 10% calcium formol for 12 hours, then were decalcified with 10% ethylene diamine tetra-acetic acid (EDTA), pH. 7.3 for 4 weeks at 4˚C. After decalcification, the specimens were rinsed with buffer solution, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Longitudinal sections of 6-7um were obtained and mounted on clean glass slides and stained with stained with Haematoxylin and Eosin stain for light microscopic examination.

3- Histological examination using ground sections:

Other right sides of the mandible were stored in buffered solution (4.6 formaldehyde solution). Each side was embedded in a quick setting transparent blocks (bioplast) and cutting sagitally, mesiodistally in a plane parallel to the long axis of the mandible by using Bronwill sectioning machine having diamond disc under water spray. The obtained longitudinal ground sections were about 1mm thick. Further thinning was carried out by Carborundum abrasive paper with water proof adhesive grit no. 320& 400 which cut slower and more evenly. When the section began to get transparent and showed signs of bending its thickness was measured using special micrometer. Ground sections of about 100 um in thickness were mounted on clean glass slides. Transverse ground sections were also obtained in the same manner. All sections were examined under light microscopy.

3. Results:

Morphological results:

Stereo-microscopic examination of sagittal sections of rabbit mandible revealed presence of one incisor, two premolar and three molar teeth. Both premolars and molars are identical in shape and are aligned vertically. Third molars are usually curved distally. The apical parts of the lower teeth are divergent like a fan (Fig. 1). The cheek teeth (premolars & molars) in the mandible are located in its center, equidistant from the incisors and the condyle. All premolars and molars consist of two longitudinal parts called laminae with characteristic apical root opening so each tooth is characterized by presence of double root openings. The pulp cavity extends to about three quarters the length of the tooth. Each pulp chamber tapers towards the occlusal end and some long thin horns arise from the pulp and extend vertically towards the periphery of the tooth (Fig. 2). The conical-shaped, pulp cavity can be visualized as bounded above and lateral by dentine which, due to continued deposition, obliterates the pulp cavity near the occlusal surface. The pulpal slits (the sites of occlusion of the pulp cavities) can be traced from the tip of the pulp chamber to the occlusal surface. The coronal portion of the pulp appeared completely obliterated by calcifications while the apical portion of the pulp was opened with some internal calcifications. The occlusal surface of the cheek teeth appeared rough and uneven due to enamel crests and dentinal grooves (Figs. 3 & 4).

II- Histological results of decalcified sections:

Light microscopic examination of rabbit lower molar tooth at 3months old showing its histological structure consisting of two laminae aligned mesially and distally to each other. Each lamina is formed of dentine and encloses a separate pulp chamber but the two chambers merge near the apical or formative end and have double apical openings. The 2 laminae are separated by a large groove which is lined by enamel towered the mesial lamina and dentine towered the distal lamina and filled by cellular cementum. Many columnar-shaped ameloblasts were observed adjacent to the cellular cementum which deposited a large amount of enamel matrix (Fig. 5). Near the apical portion of the molar teeth revealed wide pulp cavity lined by odontoblastic layer, thin layer of predentine and covered by dentine and enamel. The pulp and apical soft tissue is highly vascular, thin vascular channels course in a longitudinal direction through the apical pulp tissue were detected (Fig. 6).

In the apical region the pulp is open, wide and represents the growing portion. Single pulp chamber was appeared at the tooth apex which diverges into two pulp chamber towards the mesial and distal laminae. The pulp was displayed a range of development from undifferentiated cells and no dentine through preodontoblasts forming mantle dentine to mature secretory odontoblasts producing tubular dentine. The cervical loop was composed of a core of stellate reticulum surrounded by inner and outer enamel epithelium and appeared at the periphery of the growing end. Moreover, in the area of the folds, the enamel organ was continuous between the laminae of the tooth. Interlaminar loop are formed between the two laminae and consisting of loosely aggregated stellate reticulum in the center surrounded by inner enamel epithelium and outer enamel epithelium. The continuously growing molar of the rabbit was appeared with complex structure (Figs.7&8). The apical end of these molars is the site of differentiation and the region where initiation of odontogenesis occurs. A row of columnar odontoblasts was seen along the periphery of the pulp bordering the dentine (Fig. 8).

Histological examination of the pulp at the middle portion revealed presence of numerous denticles along the side of the pulp and an area of pulpal cell proliferation was evident. The pulp chamber was characterized by presence of normal pulp tissue and lined by odontoblastic layer that covered by dentine and enamel on its external side. While the side that facing the groove between the two laminae was covered by dentine, enamel and cellular cementum. It was obvious that, enamel layer lining of the groove was extremely thinner than enamel layer covering the external tooth surface and covered by cellular cementum. The cementum covering the cheek teeth is largely cellular. It varies in thickness around the circumference and between the subunits of the cheek teeth (Fig 9).

Other specimens revealed fusion of the small denticles forming large denticles obliterating the middle portion. Higher magnification showed that, these large denticles contained little number of dentinal tubules (Fig 10). With the narrowing of the pulp chamber, the primary dentine increased in thickness. The odontoblasts lining the pulp chamber proper gradually changed from tall columnar cells at the basal end to low cuboidal cells at the occlusal end (Figs 8-10).

The majority of cheek teeth examined had distinct incremental lines; the incremental lines were almost straight and in a near longitudinal arrangement, parallel to the odontoblast layer of the pulp. The incremental lines are almost straight and follow the wall of the pulp chamber up to the level where the odontoblasts become compressed and the pulpal slit is formed. The incremental lines are of consistent pattern throughout the length of the tooth indicating that a steady state of growth and eruption had been present (Figs. 9 &11).

Histological examination of the pulp at the coronal portion revealed complete obliteration of the pulp cavity. Most of the dentine is tubular but near the occlusal end of the tooth the central zone of the pulp filled with the dentine-included cells suggestive of an osteodentine type material. Osteodentine was widespread throughout the pulp. An irregular reparative osteodentine was deposited in the coronal pulp of rabbit molars (Fig. 11). Higher magnification revealed presence of osteodentine that contained many cell lacunae and narrow canals that contained pulp tissue making it resemble bone. The odontoblasts with other pulpal contents were seemed to be incorporated into atubular hard tissue at the occlusal end (Fig.12).

Although the morphology of the rabbit incisors was less complex than that of the molars, the structural features of the pulp and dentine were similar to those of the molars. The rabbit incisor follows the same system as the continuously growing molar. The pulp chamber contained a dental papilla which was composed of mesenchymal tissue and odontoblasts which were producing primary dentine with typical dentinal tubules. Also the histological structure of the cervical loop in rabbit incisor at labial side was consisting of inner enamel epithelium, stratum intermedium, stellate reticulum and outer enamel epithelium. Within a short distance from the cervical loop, the cells of the inner enamel epithelium had developed into ameloblasts producing enamel. On the lingual side the cervical loop is much smaller (Fig.13). Cells within the labial cervical loop are differentiating into ameloblasts and producing enamel covering the labial surface. However, the lingual cervical loop functions as a root analogue, forming epithelium root sheath of Hertwig's and anchoring the incisor in the jaw (Figs.14A&B).

The histological examination of the mandibular incisor revealed that the incisors consisted of a dentine core covered on the labial side by a thick layer of enamel and on the lingual side by thin layer of acellular cementum (Figs.15A&B). At the incisal end the pulp cavity is closed by deposition of osteodentine on its central portion and dentine on its walls. Moreover in most specimens, widespread of osteodentine formation throughout the pulp was observed, including the total occlusion of coronal portion spaces and like bone lacunae was detected in some osteodentine (Figs.15A&B).

III- Histological results of ground sections:

Histological examination of longitudinal ground sections of rabbit molar teeth revealed presence of pulp cavity space surrounded by regular tubular primary dentine at the apical portion of the tooth (Fig. 16.A), while coronal portion showed complete occlusion of the pulp cavity by dentine (Fig. 16.B). In cross section the cheek tooth of the rabbit consists of mesial and distal laminae joined by a narrow bridge of dentine on the buccal side. The distal lamina is smaller than the mesial lamina. The bilaminar cores of dentine are cover by a layer of enamel of variable thickness. External to the enamel or where the enamel is missing, external to the dentine a layer of cementum is generally present (Fig.17)

Transverse ground sections of rabbit molar at coronal portion of the mesial lamina revealed presence of numerous denticles with characteristic tubular structure within the pulp cavity space. The incremental lines denoting rhythmic matrix deposition, in dentine were numerous, well defined and appeared as dark lines, parallel to dentino-enamel junction following its contour and perpendicular to the dentinal tubules (Fig. 18). Transverse sections of the mandibular teeth were found to provide the best results.

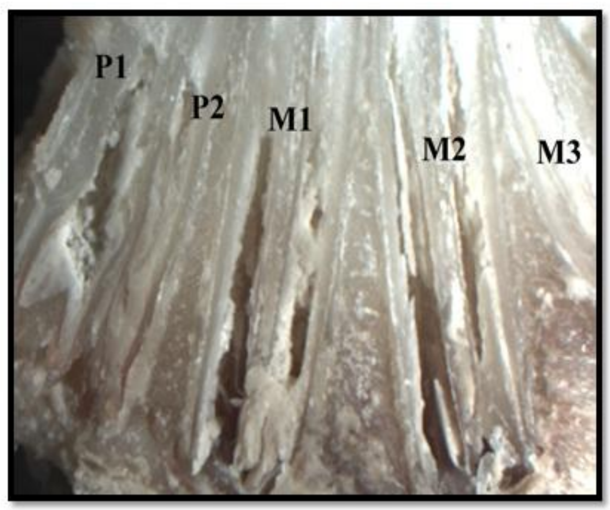

Fig (1): A photomicrograph of the sagittal section of rabbit lower cheek teeth including first premolar (P1), second premolar (P2), first molar (M1), second molar (M2), third molar (M3) and alveolar bone (arrow) **(Stereomicroscopy X 10).**

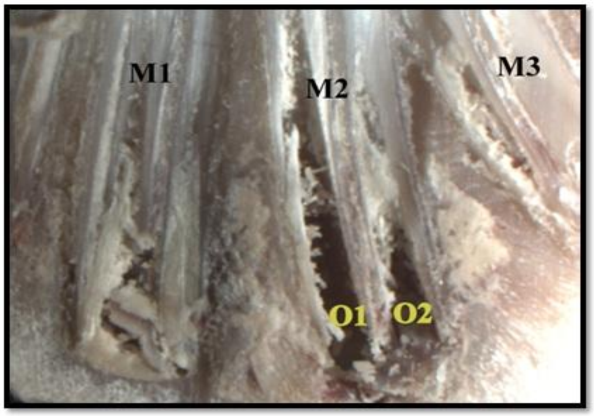

Fig (2): A photomicrograph of rabbit lower molar teeth at 3 month old showing first molar (M1), second molar (M2) and third molar (M3) with characteristic double root openings (O1 & O2), mesial lamina (arrow heads) &distal lamina (yellow arrow) (**Stereomicroscopy X 10).**

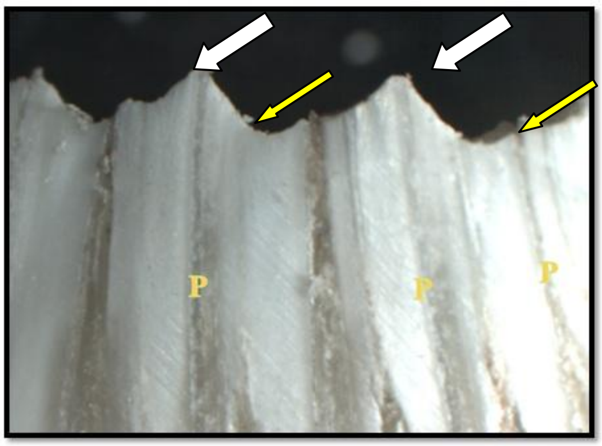

Fig (3): A photomicrograph of rabbit lower molar teeth at 3months old showing nearly completely calcified coronal portion of the pulp (pulp slit) (P), enamel crest (white arrows) and dentinal grooves (yellow arrows) (**Stereomicroscopy X 20).**

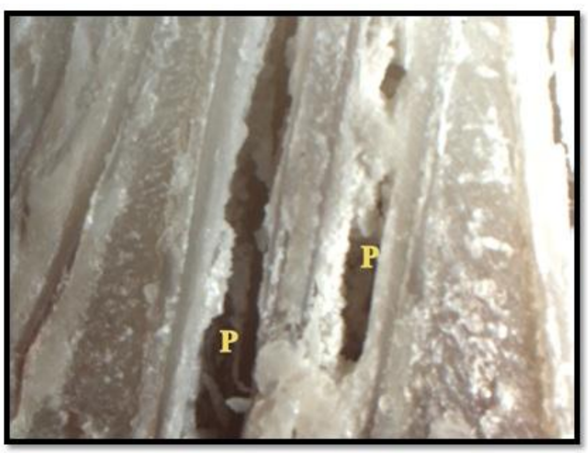

Fig (4): A photomicrograph of rabbit lower molar teeth at 3 months old showing the pulp cavity with internal dentine precipitation (P) (**Stereomicroscopy X 20).**

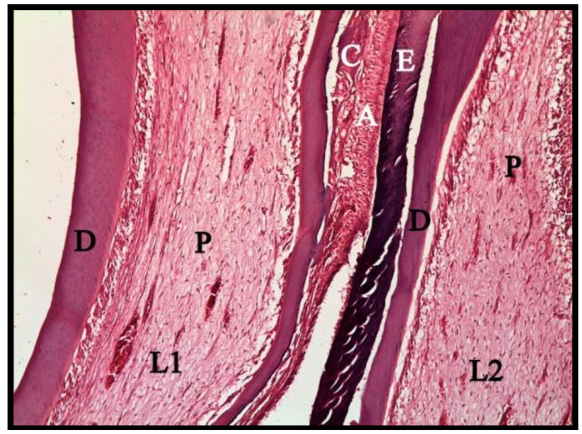

Fig (5): A photomicrograph of rabbit lower molar tooth at 3months old showing its histological structure consisting of two laminae (L1 & L2) aligned mesially and distally, each lamina is formed of dentine (D) and encloses a separate pulp chamber (P) but the 2 chambers merge apically, the 2 laminae are separated by a large groove which is lined by enamel matrix (E), ameloblasts (A) and contains cellular cement (C). (**H & E X 100).**

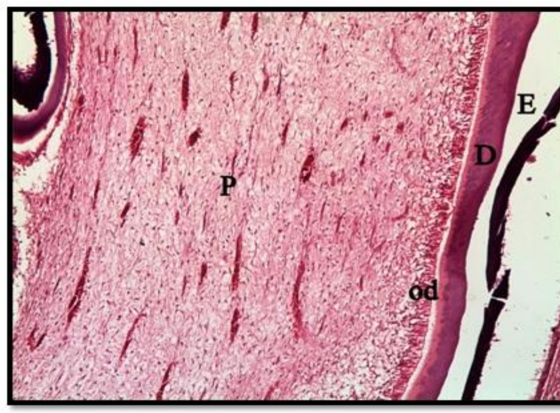

Fig (6): A photomicrograph of rabbit lower molar tooth at 3 months old showing wide pulp cavity near the apical portion (P) lined by odontoblastic layer and covered by dentin (D) and enamel (E). (**H & E X 100).**

Fig (7): A photomicrograph of rabbit lower molar tooth 3 months old at the apical odontogenic zone showing: the conical pulp cavity which appeared widest at the apex and tapering into the substance of the molar (P), cervical loop(CL) and the continuity of the enamel organ (EO) between the two forming laminae (interlaminar loop) (**H & E X 40).**

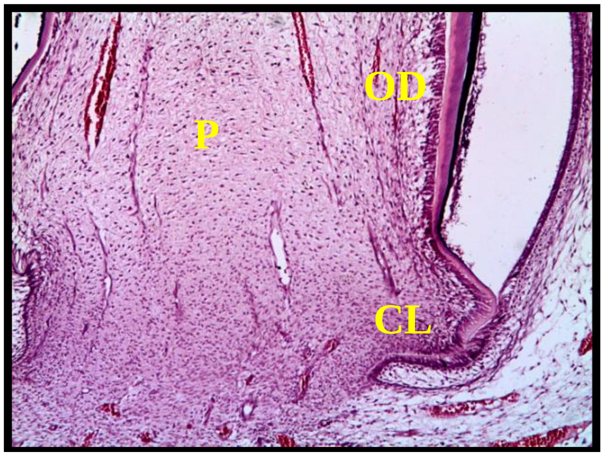

Fig (8): A higher magnification of previous photomicrograph showing the mesial region of the basal part of a laminated molar tooth of the rabbit, the dental papilla (P), a typical cervical loop(CL) with inner enamel epithelium and outer enamel epithelium, tall columnar odontoblasts (OD) in the dental papilla are depositing dentin (**H & E X 100).**

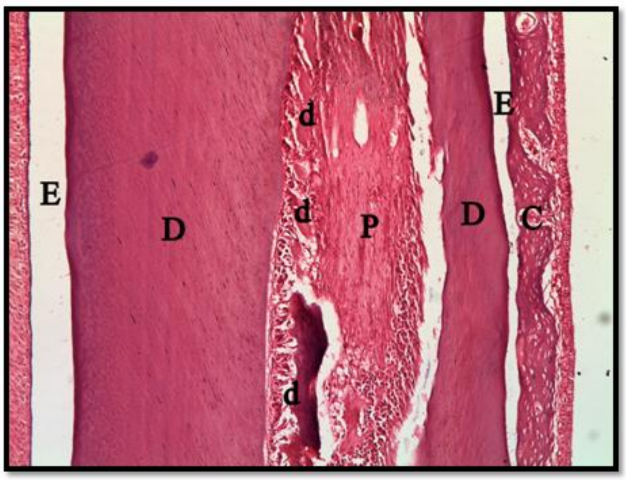

Fig (9): A photomicrograph of rabbit lower molar tooth at 3 months old showing middle portion with numerous denticles (d) along the side of the pulp (P) that covered by dentine (D), enamel (E) and cellular cementum(vascular cementum) (C). (**H & E X 100).**

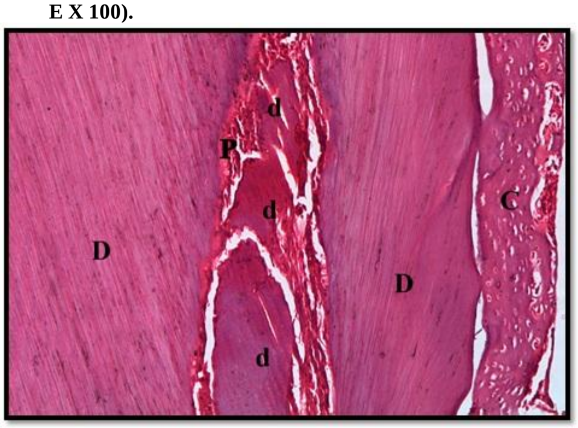

Fig (10): A higher magnification of the middle portion showing numerous large denticles formed of tubular dentine (d) along the side of the pulp (P) (**H & E X 400)**

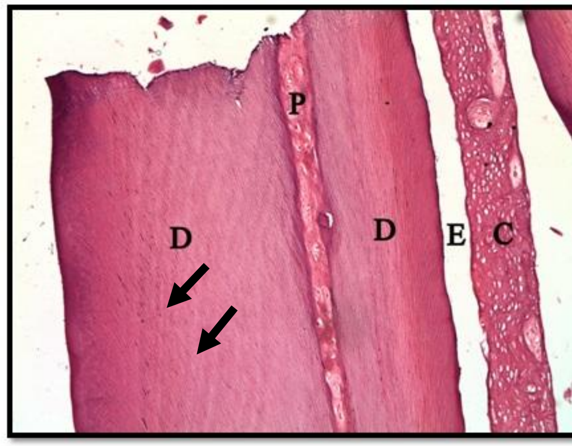

Fig (11): A photomicrograph of rabbit lower molar tooth at 3 months old showing coronal portion with completely obliterated pulp (P) with osteodentine , Note the ongitudinal incremental lines within the dentine (arrow) (**H & E X 100)**

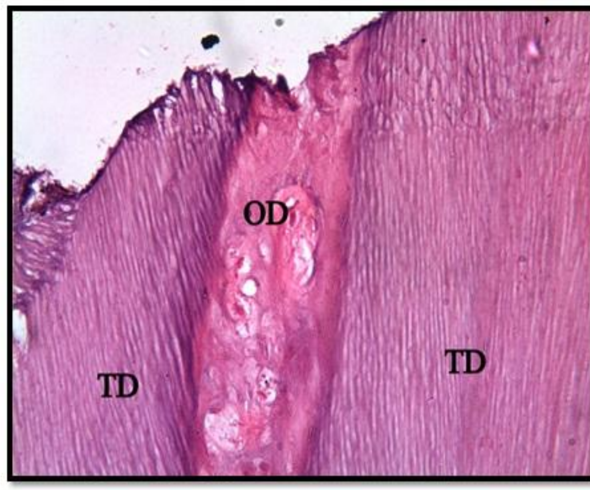

Fig (12): A higher magnification of previous photomicrograph showing complete obliteration of the pulp with osteodentine (OD) surrounded by regular tubular primary dentine (TD) (**H & E X 400)**

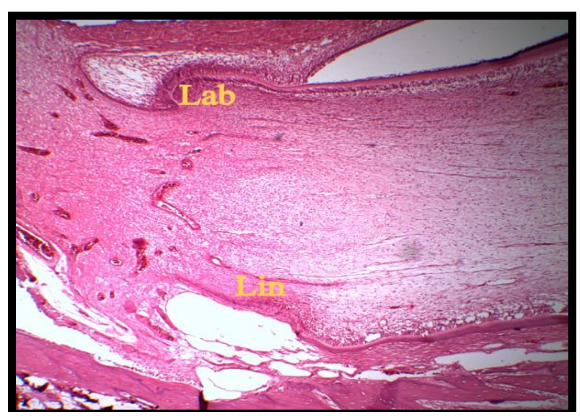

Fig (13): A photomicrograph of sagittal section of a lower rabbit incisor 3 months old at the apical portion of the pulp cavity showing the cervical loop at labial side (Lab) and cervical loop at lingual side (Lin) (**H & E X 40).**

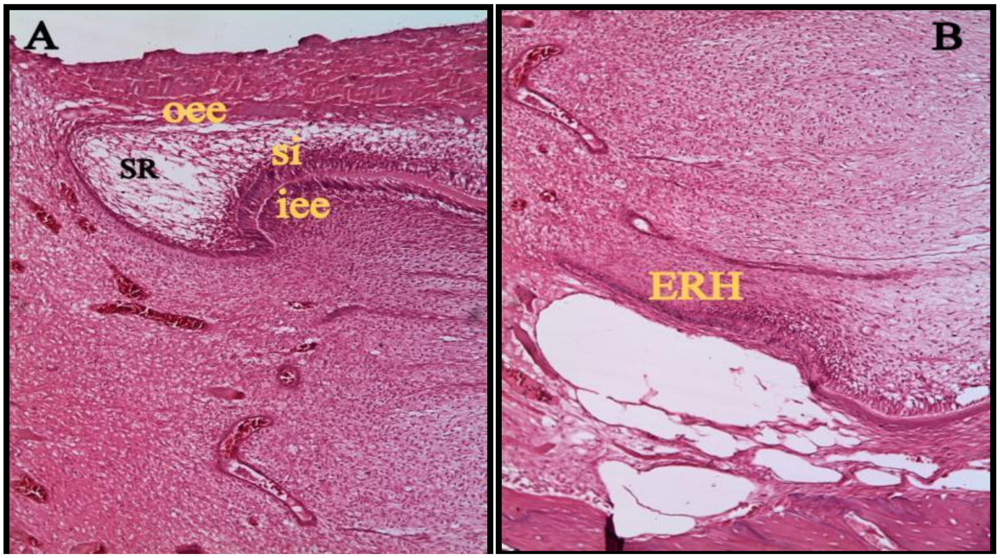

Fig (14): A higher magnification of previous photomicrograph showing (A) labial cervical loop, enamel epithelium (iee), outer enamel epithelium (oee), stratum intermedium, (si) and stellate reticulum(sr). (B) epithelium root sheath of Hertwigs(ERH) . **H & E X 100)**

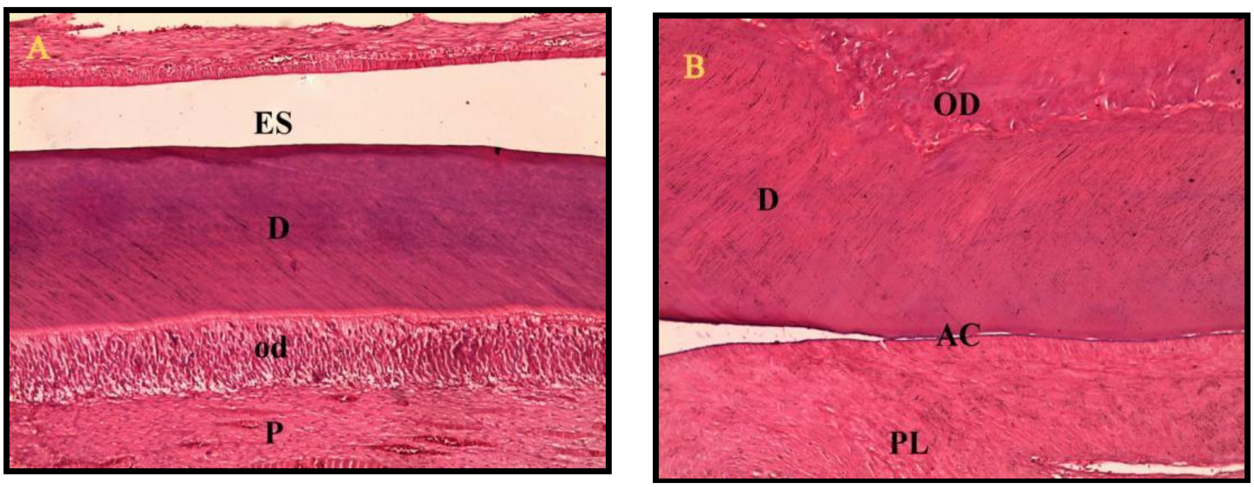

Fig (15): A photomicrograph of rabbit lower incisor tooth at 3months old **(A)** labial side showing ameloblasts (A), enamel space (E), dentine (D), predentine (Pd), odontoblasts(O) and pulp (P)**. (B)** lingual side showing periodontal ligament (PL), acellular cementum (AC), dentine(D),and osteodentine (OD) **(H & E X 100)**

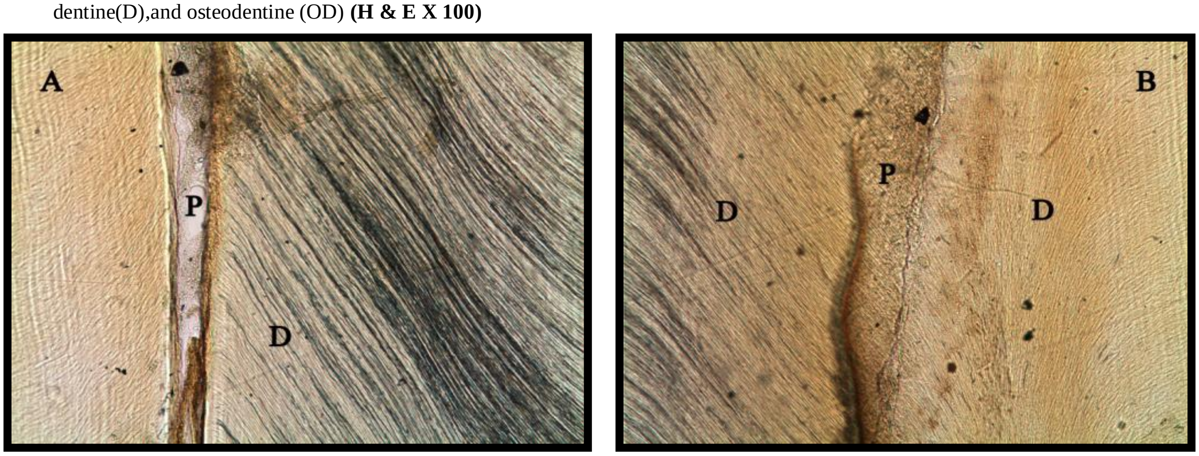

Fig (16): A photomicrograph of rabbit lower molar tooth at 3 months old showing **(A)**apical portion having pulp cavity space (P) surrounded by regular tubular dentine and **(B)** complete obliteration of coronal portion of the pulp (P) with dentine (**Longitudinal ground section X 100).**

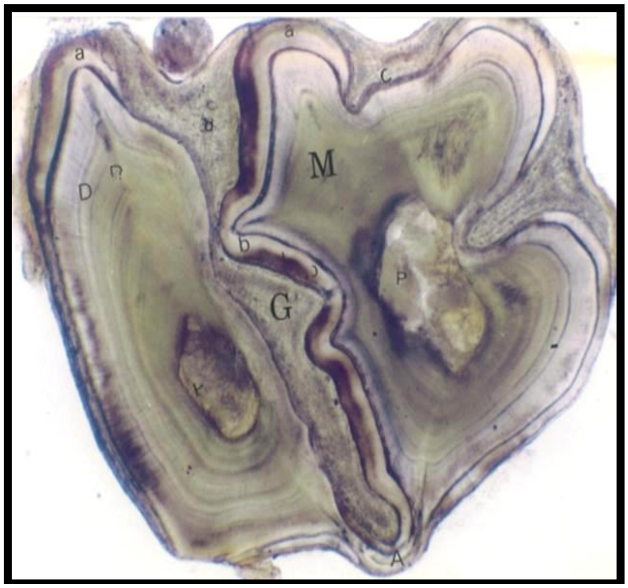

Fig (17): A photomicrograph of rabbit lower molar tooth at 3 months old showing: mesial lamina (M), distal lamina (D), narrow buccal bridge (A), external enamel (a), internal enamel(b), developmental groove (G) and cellular cementum (C). **(Transverse ground section X 25).**

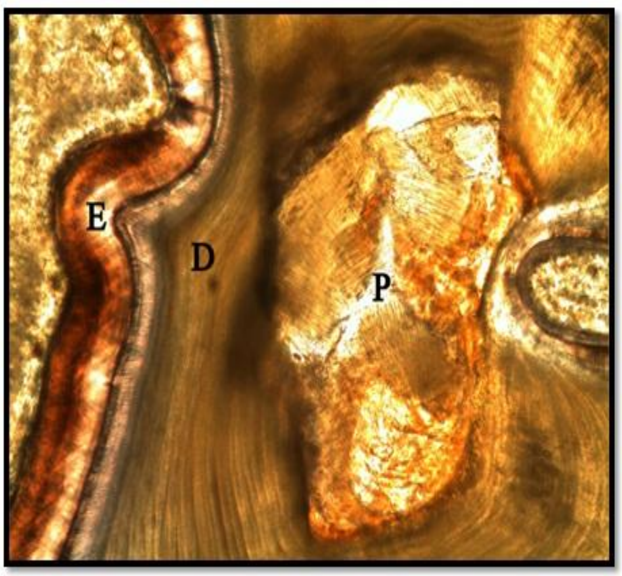

Fig (18): A photomicrograph of rabbit lower molar tooth at 3 months old showing presence of multiple tubular dentine areas (true denticles) within the pulp cavity (P) **(Transverse ground section X 100).**

4. Discussion:

The mammalian teeth can be subdivided into three groups. The first group consists of brachydont or low-crowned teeth where the root is relatively longer compared with the crown. The second group consists of hypsodont, or high–crowned teeth, where the crown is longer compared with the root. The third group consists of the hypselodont teeth, ever-growing or open-rooted teeth that grow continuously during the lifetime of the animal. They also called open rooted teeth that, refers solely to the large apical opening present in all continuously growing teeth and does not imply that the tooth actually needs to have a root in a classical form. The crown never stops growing and root formation is postponed indefinitely, but often with small tracts of dentine covered with cementum acting as regions that attaching the tooth to the jaw bone with a periodontal ligament [8].

Lagomorphs differ from other orders in the presence of peg-like second incisors directly behind the grooved upper first incisors. All teeth are hypselodont, and cheek teeth are lophodont. Rabbits have evolved ever-growing (elodont) teeth [10]. Rabbits are true herbivores. Their natural diet entails the consumption of large volumes of abrasive high fiber foods. These foods, and the volume consumed, naturally promote teeth wear. As an adaptation, rabbits have evolved ever-growing (elodont) teeth [20].

Hypsodonty is the condition of possessing high-crowned cheek teeth that are truly ever-growing. Hypsodont teeth are sometime described as ‘‘ever-growing’’ because dental epithelia remain competent to differentiate the same tissue types in the same proportions throughout the animal’s lifespan [21]. Continuously growing or hypselodont teeth could be considered as an extreme form of hypsodonty. It has been proposed that increased crown height is a relatively simple matter of delayed termination of morphogenesis/ cytodifferentiation and that hypselodonty is simply the extreme outcome of such a delay [22]. When rabbits chew, the occlusal surfaces of the molars and premolars are gradually worn away. The tissue lost during chewing is replaced by the subjacent tissue which is produced initially at the apical end and transported occlusally by continuous eruption. Tritium labeling studies suggested that in the rabbit molar the cervical loop area is the origin of the epithelial cell lineage [23].

Stereo-microscopic examination of sagittal sections of rabbit mandible revealed that the cheek teeth of rabbit consist of two longitudinal parts mesial and distal laminae with characteristic apical root opening .This results in agreement with Crossley [20] who suggested that the cheek teeth appear to be composed of three sub-units, these being fused together with cementum. However, horizontal sections and the worn occlusal surfaces revealed that the mandibular teeth are only composed of two subunits and the mesial one having a deeply invaginated enamel fold.

The structure of ever growing cheek teeth is similar to that of other truly herbivorous small species in that they are composed of multiple longitudinal subunits, each containing a pulp cavity; the subunits being fused together by vascular cementum. The cementum deposited between the folds of the tooth structure contains vascular channels. A few of these become occluded, but many remain patent [24]. The molars of guinea pigs demonstrated a unique lamination since a deep fold penetrated the substance of the tooth both on the buccal and lingual sides. Due to the lamination, the basal end of the molars demonstrated a very complex morphology. Mesially and distally, the laminae had a typical cervical loop which consisted of an outer enamel epithelium, a stellate reticulum, a stratum intermedium, and an inner enamel epithelium [25]. Guinea pigs and chinchillas have dentition very similar to rabbits from the anatomic and physiologic standpoint [26].

Histomorphological examination of the dentine pulp complex of the molar teeth of rabbits revealed presence of double root openings. Moreover the rabbit incisor appeared with wide and open pulp at the tooth apex. The lingual side of rabbit incisor is the root analogue with well developed Hertwig's epithelial root sheath and covered with dentin and cementum. The labial side is the crown analogue and covered with enamel. These open-rooted systems are considered as the main cause of continuous eruption. During classic root formation the cervical loop is created at the early morphogenesis of the crown at the cap stage and with root initiation loses its central core of stellate reticulum and stratum intermedium cells, including the putative epithelial stem cells [27-29]. A double layer of basal epithelium is left that is known as Hertwig's epithelial root sheath (HERS) [30]. As HERS proliferates, the growing epithelial sheet becomes discontinuous and forms a fenestrated network lining the root surface known as the epithelial cell rests of Malassez (ERM) [31]. Through this network the follicular mesenchyme cells can migrate to the dentine surface and differentiate into cementoblasts depositing cementum. The ERM functions in the induction of cementoblast differentiation and regulation of their function [32 & 33]. HERS forms in brachydont and hypsodont teeth when root formation is initiated and crown formation ends, and its transition to ERM is generally regarded as a typical feature of root formation. However, in continuously growing teeth a close-up of this area showed that the typical structure of the HERS, consisting only of inner and outer enamel epithelium, was not found. Instead of that the cervical loop was maintained [8].

The present study revealed that the cervical loop at the periphery of the lower cheek teeth and the labial side of the incisor was composed of a core of stellate reticulum surrounded by inner and outer enamel epithelium and appeared at the periphery of the growing end. These findings in agreement with the findings of Tummers &Thesleff [34] They reported that the cervical loop consists of several different epithelial layers. When the crown development is quite advanced the cervical loop has two developmental fate options. It can stick to being a 'crown' and continue enamel production, or it can adopt the 'root' fate. The mouse molar is an example where the entire cervical loop switches to root, whereas the vole molar only partly makes this switch, and maintains the capacity in most of its cervical loop to produce crown.

In hypselodont teeth the cervical loop is an essential structure. The continuously growing molar of the vole maintains its cervical loop during late postnatal development, and that it is present in the entire circumference of the tooth base. It has been suggested that the bulging nature caused by the large stellate reticulum compartment of the cervical loop typical of the rodent incisor is a requirement for a functional stem cell niche [29]. It is thought that the central epithelial tissue of the cervical loop, the stellate reticulum, acts as a stem cell reservoir. In continuously growing teeth such as the rodent incisor the original structure of the cervical loop is maintained and no HERS forms. The stem cells provide the epithelial progeny to sustain the continuous growth [28].

In the current study, the interlaminar loop is showed between the two laminae and consisting of loosely aggregated stellate reticulum in the center surrounded by inner enamel epithelium and outer enamel epithelium . The continuously growing molar of the rabbit was appeared with complex structure. This finding confirms the result of Caton & Tucker [35] they found that the continuously growing molar of the sibling vole has a complex structure. The vole and mouse are both rodents and are closely related species but the vole molar maintains the crown fate and will grow continuously. The epithelium has folded several times into the mesenchyme in a complex manner. These folds run almost all the way to the base of the tooth and create large epithelial compartments that run deep down into the dental mesenchyme. These compartments consist of a basal layer of inner enamel epithelium enclosing a large compartment of stellate reticulum and stratum intermedium. Structurally the intercuspal loop therefore resembles the cervical loop. The cervical loop is a structure that is not restricted to one position but is found in the circumference of the entire base of the tooth.

Histological examination of rabbit incisor revealed the present of large cervical loop at the labial side. This finding was in agreement with those of Kawano *et al.*, [36]and Tummers & Thesleff [12] they found that, in the mouse incisors (elodont teeth) only the labial cervical loop functions to generate ameloblasts that produce enamel on the labial tooth surface. The asymmetric deposition of enamel results in the sharpening of the mouse incisor by single face erosion.

Histological examination of the pulp chamber revealed continuous deposition of dentine to such an extent that, in the coronal portion of the teeth the pulp chamber was almost completely occluded. Therefore, the pulp diminished, and the whole tooth looked like an irregular dentin lump with remnants of pulp tissue in the center. This could be considered as adaptive function for teeth protection and preservation of teeth vitality. It was also postulated that continuously growing teeth are an animal's adaptation to the extensive wear of its dental apparatus, which could result in loss of life because of premature loss of teeth [37]. There is normally rapid wear of the cheek teeth, around 3mm per month in a wild rabbit [38].

The rate of growth of normal elodont teeth and the shape of their pulp cavities are regulated by the rate of eruption while eruption is largely regulated by the rate of attrition [39]. As elodont teeth grow and erupt the odontoblasts lining the pulp cavities deposit new dentine matrix. The rate of dentine deposition on the walls of the pulp cavity in the elodont teeth of rats and rabbits is generally around 16 microns per day [40].

In the present study the examination of incisors and molar of the rabbit revealed that the calcified tissues in the pulp seem to move toward the occlusal end with the eruptive movement of the teeth and to be worn out eventually. New pulpal tissues generate at the base to replace the calcified materials. Tubular dentine is produced at the apical end and becomes thicker as it moves occlusally. The constant deposition of dentine in rabbit teeth normally prevents pulpal exposure on the occlusal surface as it is worn away by normal attrition. Dentine deposition in most mammals occurs in an appositional manner throughout life. In contrast to the slow incremental circumpulpal deposition of primary and secondary dentine, reparative dentine is believed to be a localized defensive response by the pulp which deposits dentine at specific sites in response to dentinal irritation, although the mechanism controlling its formation[41].

Dentin is laid down incrementally throughout life in the ever-growing incisors of rodents and lagomorphs, with each increment forming a cone-shaped deposit along the pulp cavity. The constant deposition of secondary dentine in rabbit teeth normally prevents pulpal exposure on the occlusal surface as it is worn away because of normal attrition [42]. Moreover teeth provide a record of their formation in their incremental growth and mineralization lines, so histological sections show the history of the teeth. The clinically normal cheek teeth had long thin pulp cavities with a uniform internal structure [43].

Bishop [44] reported that most of the dentine in rabbit teeth is tubular dentine and near the occlusal end, postodontoblasts deposit an atubular tissue which closes the pulpal ends of the tubules and the pulpal contents degenerate and become embedded in the forming atubular tissue. Author added that in rabbit teeth the atubular tissue represents the pulp's reaction to the physiological trauma of mastication at the occlusal surface and prevent direct communication between the occlusal surface and the vital parts of the pulp.

Osteodentine formation was detected in the coronal portion of the rabbit molar teeth. The formation of osteodentine began at the periphery of the pulp and gradually advanced towards the central region. Within the osteodentine, narrow canals that contained pulp tissue as well as many cells were observed. Osteodentine formation was also detected in continuously erupting teeth of guinea pigs in the coronal halves of both incisors and molar. Pulp in guinea pig incisors and molars have revealed a bone-like tissue (osteodentine) with the coronal pulp containing anastomosing blood vessels. In the parts of the pulp where osteodentine was being formed cells become trapped in this irregular dentin. Surrounding the osteodentine there was a layer of normal dentin. In extreme cases, most of the occlusal part of the tooth was composed of osteodentine with only a thin layer of normal dentin adjacent to the enamel [25].

Rabbit's permanent teeth develop a substantial amount of cementum over their enamel surface. Its formation follows the loss of continuity of the reduced enamel epithelium. The cementum which forms within the developmental groove of these teeth contains many cellular inclusions and is of a fibrillar nature that contains recognizable collagen fibrils in its organic matrix. The cementum on the periphery of the crowns, particularly of permanent teeth, is primarily acellular and consists of fibrillar and afibrillar cementum, the latter containing no recognizable collagen fibrils in its organic matrix. Degeneration of some cellular elements of cellular cementum as a result of continuous eruption is accompanied by the appearance of dense bodies resembling lysosomes and autophagic vacuoles within these cells. These changes are not observed in connection with the disappearance of reduced enamel epithelial cells prior to the onset of cementogenesis [45]

In conclusion, rabbit molar teeth are continuously growing and subjected to continuous wear out of the tooth structure. The pulp cavity reveled precipitation of different types of dentine. Middle portion showed presence of large denticles having little number of dentinal tubules while, coronal portion revealed complete obliteration of the pulp chamber with osteodentine.

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References:

1-Wiggs B, Lobprise H.(1995): Dental anatomy and physiology of pet rodents and lagomorphs, in Crossley DA, Penman S (eds):

Manual of Small Animal Dentistry, ed 2. Cheltenham, British Small Animal Veterinary Association; 68–73.

2- Hirschfeld Z, Weinreb M, Michaeli Y. (1973): Incisors of the Rabbit: Morphology, Histology, and Development. J. Dent. Res.; 52: 377.

3- Crossley D. (1995): Clinical Aspects of Lagomorph Dental Anatomy: The Rabbit (Oryctolagus cuniculus). J. Vet. Dent.;12 (4): 137-140.

4-Navarro J.A, Sottovia-Filho D, Leite-Ribeiro M.C, Taga R.(1976):Histological study on the postnatal development and sequence of eruption of the mandibular cheek-teeth of rabbits (Oryctolagus cuniculus). Arch Histol Jpn.;39 (1):23-32.

5- Kertesz P. (1993): Veterinary Dentistry and Oral Surgery. London: Wolfe Pb l; 36-37.

6- Crossley D.A. (2001): Dental disease in chinchillas in the United Kingdom. J. Small Animal Practice 42, 12-19.

7- Verhaert L. (2004): Dental diseases in lagomorphs and rodents. In: Gorrel C. (editor). Veterinary Dentistry for the General Practitioner. Elsevier Health Science, Edingburgh, p. 175-196.

8- Capello V, Gracis M. (2005): The anatomy of the skull and teeth. In: Capello V., Gracis M., Lennox A.M. (editors): Rabbit and Rodent Dentistry Handbook. Zoological Education Network, Florida, 3-17.

9- Tims H.W.M, Henry C.B. (1923): Tomes' Dental Anatomy, 8th ed, London: J. & A.Churchill, , 427-430.

10- Harcourt-Brown F.(2002): Dental disease. In *Textbook of rabbit medicine*. Oxford, UK: Butterworth-Heinemann.;165-206

11- Michaeli Y, Hirschfeld Z, Weinreb M. (1980): the cheek teeth of the Rabbit: Morphology, Histology, and Development. Acta anat. 106:223-239.

12- Tummers M, Thesleff I. (2008): Observations on continuously growing roots of the sloth and the K14-Eda transgenic mice indicate that epithelial stem cells can give rise to both the ameloblast and root epithelium cell lineage creating distinct tooth patterns . Evolution & development; 10: 2, 187–195.

13- Ryšavy R. (2007): The Missouri House Rabbit Society, hosted by the Kansas City Missouri House Rabbit Society. Page accessed 9.en. Wikipedia.org/ wiki/ tooth.

14- Lobprise H.B, Wiggs R.B. (1991). Dental and Oral Disease in Lagomorphs. J. Vet. Dent. 8, 11-17.

15- Harcourt-Brown F.M. (1995): A review of clinical conditions in pet rabbits associated with their teeth. The Veterinary Record 137, 341-346.

16- Meredith A. (2006): Rabbit medicine. In: *Proceedings Twiejo*, 21th ed, Gent, p. 23-24.

17- Bhaskar S N. (1980): Orban's oral histology and embryology. 9th ed. St. Louis; CV Mosby;

18- Nakashima M, Akamine A. (2005): The application of tissue engineering to regeneration of pulp and dentin in endodontics. J Endod; 31: 711-718.

19- Smith A.J, Lumley P.J, Tomson P.L, Cooper P.R. (2008): Dental regeneration and materials: a partnership. Clin Oral Investig; 12: 103-108.

20- Crossley DA. (2003): Oral biology and disorders of lagomorphs. Vet Clint Exit Anim.; 6:629-659

21- Mones A. (1982): An equivocal nomenclature: what means hypsodonty? Pala¨ontologische Zeitschrift 56: 107–111.

22- Jernvall J, Fortelius M. (2002): Common mammals drive the evolutionary increase of hypsodonty in the Neogene. Nature 417, 538-540.

23-Starkey W. E. (1963): The migration and renewal of tritium labeled cells in the developing enamel organ of rabbits. Br. Dent. J.; 115: 143–153.

24- Oxberry B.A. (1975): An anatomical, histochemical and autoradiographic study of the ever-growing molar dentition of Microtus with comments on the role of structure in growth and eruption. J.Morph.147, 337-350.

25-Holmstedt JO, Mc Clugage SG, Clark JS, Guevara M J. (1977): Osteodentin formation in continuously erupting teeth of guinea pigs. J Dent Res.; 56(12):1569-1576.

26- Reiter AM. (2008): Pathophysiology of dental disease in the rabbit, guinea pig and chinchilla. JEPM, 17(2): 70-77.

27-Ten Cate A. R.(1961): Recruitment in the internal enamel epithelium as a factor in growth of the human tooth germ. Br. Dent. J.; 110: 267–273.

28-Harada H, Kettunen P, Jung H. S, Mustonen T, Wang Y. A, Thesleff, I.(1999): Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J. Cell Biol.; 147: 105–120.

29- Harada H, Toyono T, Toyoshima K, Yamasaki M, Itoh N, Kato S. Sekine K, Ohuchi, H. (2002): FGF10 maintains stem cell compartment in developing mouse incisors. Development 129, 1533-1541

30-Thomas H. F.(1995): Root formation. Int. J. Dev. Biol.; 39: 231–237.

31- Ten Cate A. R. (1996): The role of epithelium in the development, structure
and function of the tissues of tooth support. Oral. Dis.; 2: 55–62.

32-Bosshardt D, Schroeder H. E. (1996): Cementogenesis reviewed: a comparison between human premolars and rodent molars. Anat. Rec.; 245: 267–292.

33- Kagayama M, Sasano Y, Zhu J, Hirata M, Mizoguchi I, Kamakura S. (1998): Epithelial rests colocalize with cementoblasts forming acellular cementum but not with cementoblasts forming cellular cementum. Acta Anat.; 163: 1–9.

34- Tummers M, Thesleff I. (2003): Root or crown: a developmental choice orchestrated by the differential regulation of the epithelial stem cell niche in the tooth of two rodent species. Development, 130, 1049-1057

35-Caton J, Tucker A. S. (2009): Current knowledge of tooth development: patterning and mineralization of the murine dentition**.** J. Anat., 214: (4),502–515

36- Kawano S, Saito M, Handa K. (2004): Characterization of dental epithelial progenitor cells derived from cervical-loop epithelium in a rat lower incisor. J Dent Res.;83: 129-133.

37- NESS A. R. (1960): Continuously Growing Teeth, Discovery.; 21: 488-491.

38- Crossley D. (2001): Risk of pulp exposure when trimming rabbit incisor teeth. Proceedings of the 10th European Veterinary Dental Society Annual Congress, Berlin, 175-196.

39-Risnes S, Septier D, Goldberg M. (1995): Accelerated eruption of rat lower incisor. Relationship between impeded and unimpeded eruption rates, rate of attrition, tooth length, and production of dentin and enamel. Connective Tissue Research 32, 183-189.

40- Ahlgren S.A. (1969): Rate of apposition of dentine in upper incisors of normal and hormone treated rats. Acta Orthopaedica Scandinavia Supplement 116, 22 - 111

41- Sicher H, Bhaskar S. N. (1972): Orban's Histology and Embryology. 7th edition p. 116. The C. V. Mosby Company. Saint Louis.

42- Schour I, Steadman S. R. (1935): The growth pattern and daily rhythm of the incisor of the rat. Anatomical Record 63:325–333.

43-Suckling G.W. (1989): Developmental defects of enamel - Historical and present day perspectives of their pathogenesis. *Advances in Dental Research* 3, 87-94.

44- Bishop M. A.(1995): Is rabbit dentine innervated? A fine-structural study of the pulpal innervation in the cheek teeth of the rabbit
J. Anat. 186, pp. 365-372,

45- Listgarten M, Kamin A. (1969): The development of a cementum layer over the enamel surface of rabbit molars—A light and electron microscopic study. Archives of Oral Biology; 14(8): 961- 972.

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