PULP TISSUE CHANGES DURING SHEDDING OF THE DECIDUOUS TEETH IN DOGS (HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURE STUDY)

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ABSTRACT

Background: The resorption of the roots of deciduous teeth is a physiological phenomenon. Different histological studies have been reported concerning the tissue changes involved in the physiological root resorption and shedding of deciduous teeth in various animals.

Methods: 5 young (2 months) male mongrel dogs were used in this study. After sacrifice, the lower jaws were dissected out and the parts of the jaws with the third deciduous premolars were dissected out followed by fixation and decalcification. The right sides were prepared for histological and Fas immunohistochemical examination, while the left sides were prepared for transmission electron microscopic examination.

Results: Histological examination of the pulp of deciduous third premolars during shedding showed resorption of the internal dentin surface with entrapped odontoclasts. There were thick collagen bundles and dilated blood vessels engorged with red blood cells. Extravasated red blood cells and chronic inflammatory cells were detected apically. Areas of reparative dentin were found. Electron microscopic examination revealed the ultrastructure of odontoclasts. Macrophages, lymphocyts, plasma cells, neutrophils and monocytes were detected as well as myelinated nerves. Many cells revealed characteristic nuclear surface blebbing and apoptotic nulclear morphology. Active fibroblasts as well as fibroclasts were detected. Immunohistochemical examination revealed positive Fas immunoreactivity in all pulp cells and in odontoclasts indicating apoptosis.

Keywords: shedding; odontoclasts; ultrastructure; apoptosis; inflammatory cells.

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INTRODUCTION

The resorption of the roots of deciduous teeth is a physiological phenomenon. This resorption is usually initiated on the side of the root of deciduous teeth nearest the permanent successor at a given time. It is generally believed that the pressure from erupting permanent teeth, mechanical-occlusal trauma and inflammatory processes are all considered as contributing factors in the shedding process [1&2].

Numerous histological studies have been reported concerning the tissue changes involved in the physiological root resorption and shedding of deciduous teeth in various animals. The dental hard tissues are physiologically resorbed by odontoclasts that were differentiated from tartrate resistant acid phosphatase (TRAP) positive mononuclear cells, which presumably originated from circulating progenitor cells. Then membrane specialization of odontoclasts in the form of development of a clear zone and ruffled border were induced following their contact with the resorption surface. Multinucleation of odontoclasts occurred only after their attachment to the resorption surface. The mature multinucleated odontoclasts resorbed predentin as well as dentin. At the end of the resorption, odontoclasts gradually lose their ruffled borders and become detached from the resorbed surface [3].

The dental pulp is a specialized connective tissue with a peculiar organization and location. It is surrounded by dentin, which is a hard dental tissue, and presents fibroblasts, odontoblasts, endothelial cells, collagen fibers, glycosaminoglycans, lymphocytes, macrophages, bloods vessels and nerves[4]. During the physiological root resorption in primary teeth, the pulp is also eliminated. Most studies have focused only on the resorption of hard dental tissues however; the sequence of events and the mechanism responsible for the physiological resorption and elimination of the pulp cells remain unclear [5-8].

So that, the aim of the present study was to demonstrate the histological and ultra structural changes of the pulp tissue during physiological shedding of the deciduous teeth as well as localization of Fas immune reactivity that indicates apoptosis.

MATERIAL AND METHODS

5 young male mongrel dogs about two months old were used in this study. After sacrifice, the lower jaws were dissected out and the parts of the jaws with the third deciduous premolars were dissected out followed by fixation in glutaraldehyde and decalcification in 10% ethylene diamine tetra acetic acid (EDTA) in 0.1 M sodium cacodylate buffer (pH7.4) for 4 weeks at 4°C.

I- The decalcified parts of the right sides were washed by tap water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections of 6-7 um were obtained and mounted on clean glass slides and stained with Haematoxyline and Eosin stain for histological examination using light microscope.

II- 5um thick sections were cut and mounted on poly-L-lysine coated glass slides and prepared for Fas immunohistochemical staining for detection of apoptotic changes in the pulp.

III- The decalcified parts of the left sides were cut into small fragments of about 1×1 mm3 using a very sharp blade then fixed in a solution prepared by mixing equal volumes of 3% glutaraldehyde and 0.1 phosphate buffer (pH= 7.4) at 4°C for 3-5 hours. Specimens were rinsed in 0.1% phosphate buffer, post fixed with 1% osmium tetroxide and 0.1 phosphate buffer then rinsed with distilled water. Then the specimens were dehydrated in ascending grades of ethanol, infiltrated with resin

and embedded into araldite resin capsules. Semi thin sections 0.5 um thick were cut and fixed on glass slides then stained by Toulidine Blue for light microscopic examination to select the best areas to be examined by the electron microscope. Ultra thin sections 60–100 A° thick were cut and stained with Uranyl acetate then with Lead citrate and examined by **(Joel 100 S transmission electron microscope)** at different magnifications and photographed using **C.C.D camera** in the electron microscope unit at the National Institute of Cancer, Cairo University.

RESULTS

Histological results

Light microscopic examination of the third deciduous premolars during shedding revealed resorption of the internal dentin surface in the crown as well as the root. The coronal dentin appeared resorbed with numerous entrapped odontoclasts and characteristic scalloped line of resorption. The odontoblastic layer and the predentin were disappeared (fig.1). The apical part of the root also revealed resorption of the internal dentin surface with numerous entrapped odontoclasts and characteristic deep scalloped line of resorption. The pulp tissue at the apical area demonstrated heavy infiltration of chronic inflammatory cells (fig. 2).

The pulp core showed numerous dilated blood vessels engorged with red blood cells and degenerated collagen fibers (fig. 3). Some thick walled blood vessels and a lot of vacuoles were also detected (fig. 4). Some specimens revealed formation of localized areas of reparative dentin under the cusp tips and were lined by thin layers of predentin. The odontoblastic layer was degenerated. The underlying pulp tissue was also resorbed and had scalloped margin (fig. 5).

Ultrastructure results

Electron microscopic examination of the pulp tissue of the 3rd deciduous premolars during shedding revealed the ultrastructure of odontoclasts. These cells appeared large, multinucleated and having numerous cytoplasmic vacuoles (fig. 6). Ultrastructure examination also demonstrated presence of two types of fibroblasts. Active formative cells (fibroblasts) having large elongated nucleus, well developed rough endoplasmic reticulum and numerous Golgi. It was

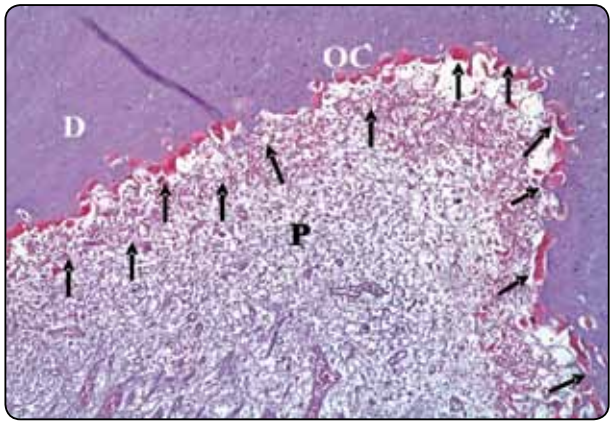

Fig. (1): A photomicrograph of deciduous 3rd premolar during shedding showing resorption and scalloping of the internal surface of coronal dentin (D) with numerous entrapped odontoclasts (OC & arrows) and complete loss of the odontoblastic layer (Orig.mag. X 100).

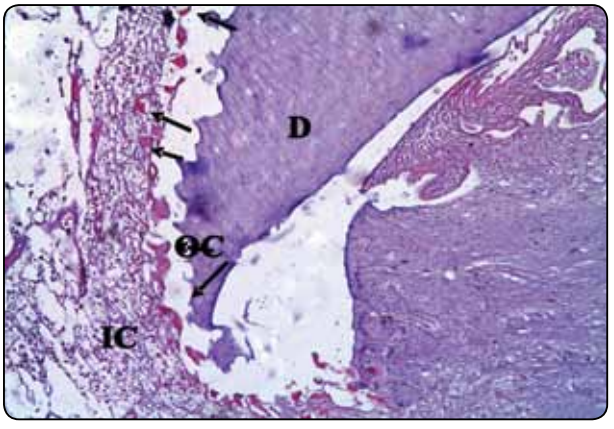

Fig. (2): A photomicrograph of deciduous 3rd premolar during shedding showing resorption and deep scalloping of the internal surface of apical dentin (D) with numerous entrapped odontoclasts (OC & arrows) and heavy infiltration of pulp tissue with chronic inflammatory cells (IC) at the apical end of the tooth (H & E Orig. mag. X 100).

surrounded by transverse and longitudinal sections of collagen fibers (fig.7). The other type is resorptive fibroblast (fibroclast) that appeared large with elongated nucleus and having numerous primary and secondary lysosomes. It was surrounded by degenerated collagen fibers (fig. 8).

Electron microscopic examination of the pulp tissue during shedding revealed presence of different types of inflammatory cells including macrophages, monocytes, neutrophils(PNL), lymphocytes and plasma cells. Macrophages appeared as large cells having numerous secondary lysosomes (phagosomes) and numerous pseudopodia reflecting their phagocytic ability and amoebic movement (fig. 9). Monocytes appeared as large cells with segmented nucleus and numerous small pseudopodia extending from the cell. There were numerous extravasated erythrocytes (fig. 10). Neutrophils (polymorphonuclear leucocytes) were also detected with their characteristic lobulated nuclei and numerous mitochondria (fig. 11). Plasma cells were detected with their characteristic nuclear morphology of cart

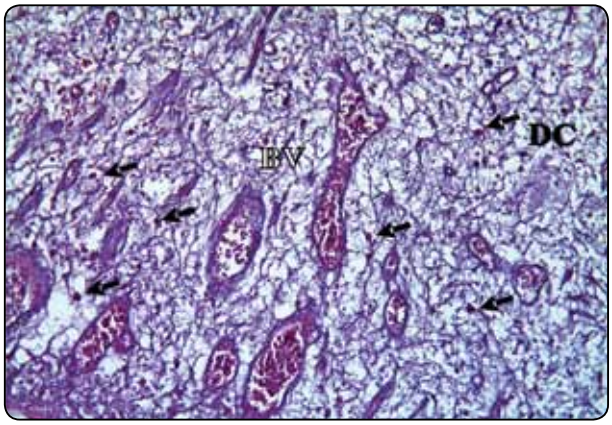

Fig. (3): A photomicrograph of the pulp tissue during shedding showing numerous dilated blood vessels (BV) engorged with red blood cells, degenerated collagen fibers (DC) and extravasater RBCs (arrows) (H & E Orig.mag. X 200).

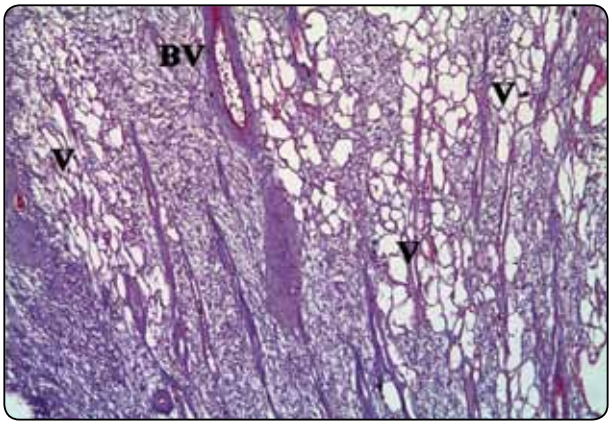

Fig. (4): A photomicrograph of the pulp tissue during shedding showing numerous large vacuoles (V) and thick wall blood vessel (BV) (H & E Orig.mag. X 100)

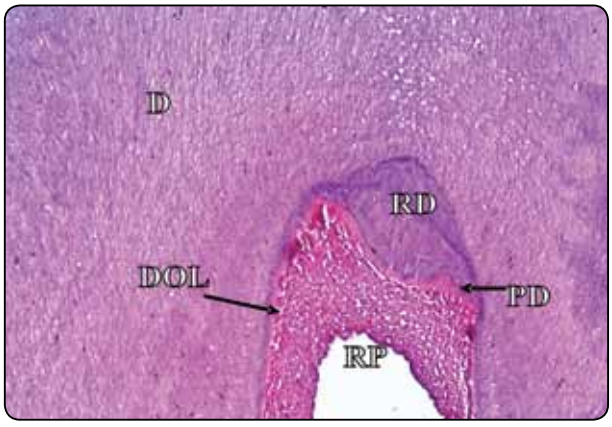

Fig. (5): A photomicrograph of the deciduous 3rd premolar during shedding showing localized area of reparative dentin (RD) at the cusp tip, predentin (PD) and degenerated pulp tissue (DP) and degenerated odontoblastic layer (DOL) (H & E Orig.mag. X 100).

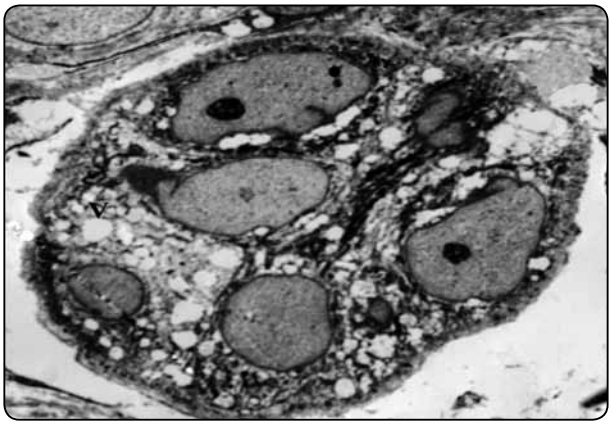

Fig. (6): Electron micrograph showing the ultrastructure of odontoclasts having multiple nuclei (N) and numerous vacuoles (V) (Uranyl acetate & Lead citrate x 1000).

wheel appearance and it was surrounded by degenerated collagen fibers (fig. 12). The pulp tissue also revealed numerous apoptotic cells as well as normal cells. There were numerous apoptotic lymphocytes and apoptotic monocytes. Numerous extravasated erythrocytes were detected and macrophages engulfing these erythrocytes in their cytoplasm were seen (fig. 13). Surface blebbing of the apoptotic cells and the characteristic apoptotic nuclear morphology with the formation of apoptotic bodies were clarified (figs. 14 & 15). Ultrastructure examination also revealed presence of numerous sections of myleinated nerve fibers with encircling Schwann cells (fig. 16).

Immunohistochemical results

Immunohistochemical examination of the 3rd deciduous premolars during shedding revealed intense positive immunoreactivity for Fas in all pulp cells (Fig. 17). The entrapped odontoclasts along the internal dentin surface also showed positive Fas immunoreactivity (Fig. 18).

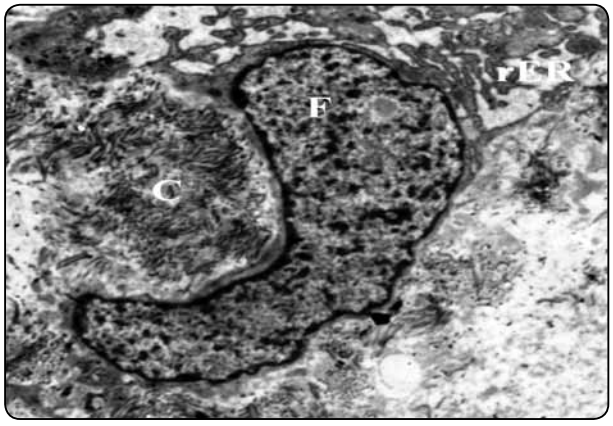

Fig. (7) Electron micrograph showing active fibroblast (F) having large elongated nucleus with well developed rough endoplasmic reticulum (rER) and Golgi surrounded by transverse and longitudinal sections of collagen fibers (C) (Uranyl acetate & Lead citrate x 2000).

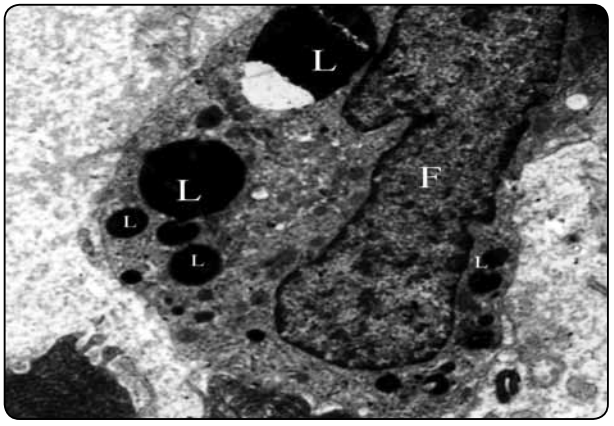

Fig. (8): Electron micrograph showing resorptive fibroblast (fibroclast) (F) with primary and secondary lysosomes (L) and surrounded by degenerated collagen (DC) (Uranyl acetate & Lead citrate x 2000).

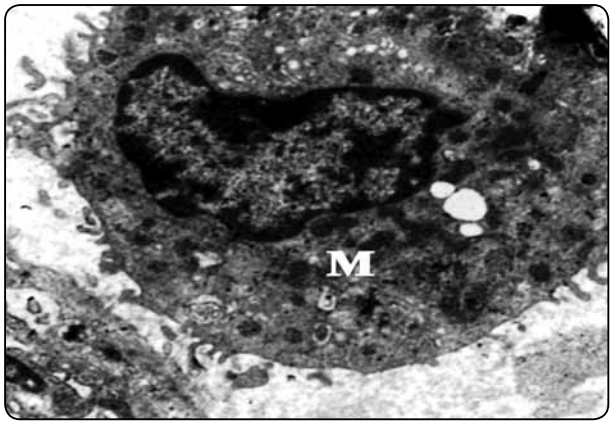

Fig. (9): Electron micrograph showing an active Macrophage (M) with numerous secondary lysosomes and pseudopodia reflecting phagocytic ability and amoebic movement (Uranyl acetate & Lead citrate x 3000).

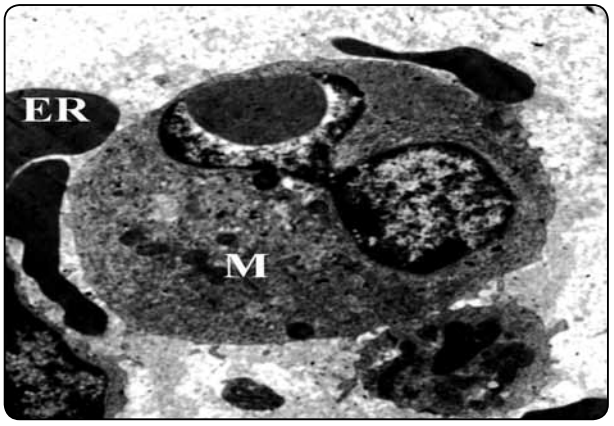

Fig. (10): Electron micrograph showing Monocyte (M) with segmented nucleus small pseudopodia extending from the cell and surrounded by extravasated erythrocytes (Uranyl acetate & Lead citrate x 2000).

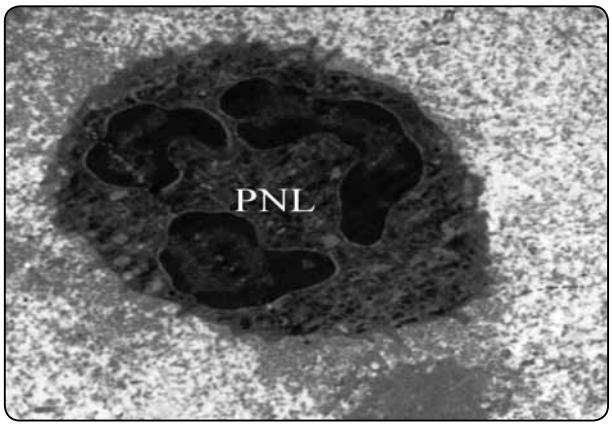

Fig. (11): Electron micrograph showing Neutrophil (PNL) with its characteristic lobulated nucleus surrounded by degenerated collagen (Uranyl acetate & Lead citrate x 4000).

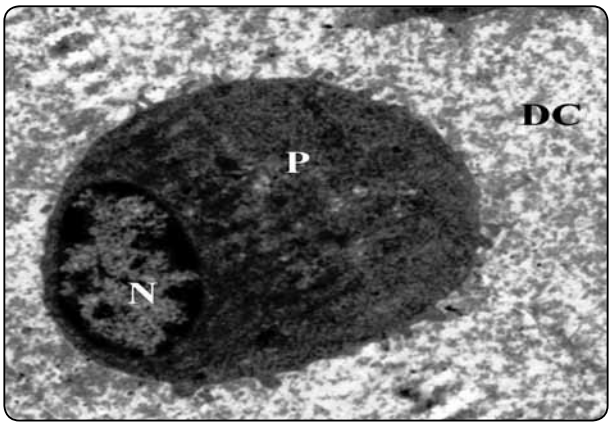

Fig. (12): Electron micrograph showing Plasma cell (P) with an eccentric oval nucleus (N) with the chromatin coarsely clumped in a characteristic "cart-wheel" pattern and surrounding degenerated collagen (DC) (Uranyl acetate & Lead citrate x 2000).

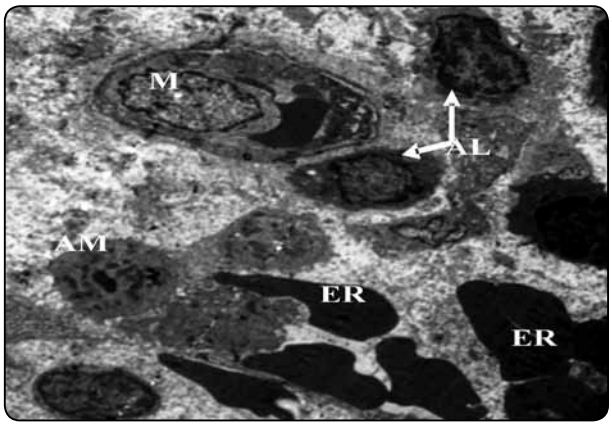

Fig. (13) Electron micrograph showing extravasated erythrocytes (ER) in-between apoptotic lymphocytes (AL) and apoptotic monocytes (AM) and Macrophage engulfing erthrocytes in its cytoplasm (M). (Uranyl acetate & Lead citrate x 1000).

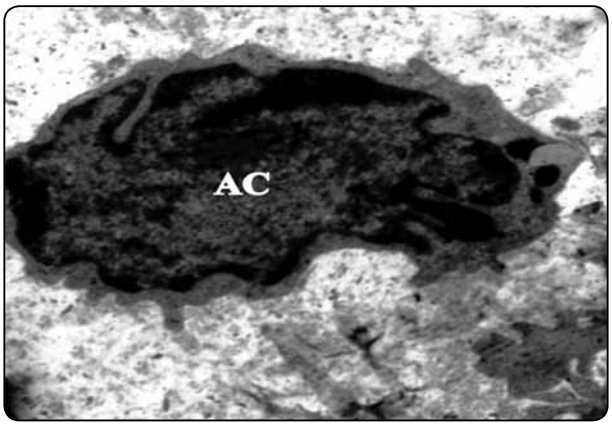

Fig. (14): Electron micrograph showing characteristic surface blebbing and apoptotic nuclear morphology (AC) of one of the pulp cells (Uranyl acetate & Lead citrate x 3000).

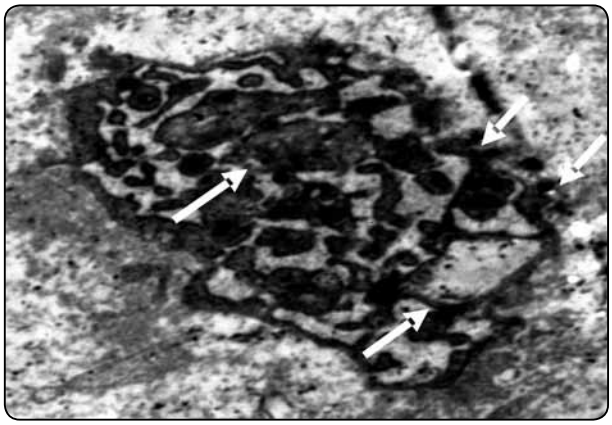

Fig. (15): Electron micrograph showing characteristic apoptotic nuclear morphology of one of the pulp cells with formation of apoptotic bodies (arrows) (Uranyl acetate & Lead citrate x 3000).

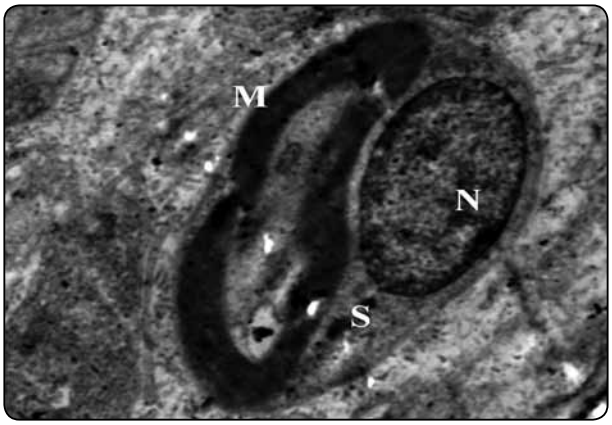

Fig. (16): Electron micrograph showing a myelinated nerve fiber (M) of the pulp is sectioned transversely at the level of the nucleus (N) of an ensheathing shwann cell (S) (Uranyl acetate & Lead citrate x 3000).

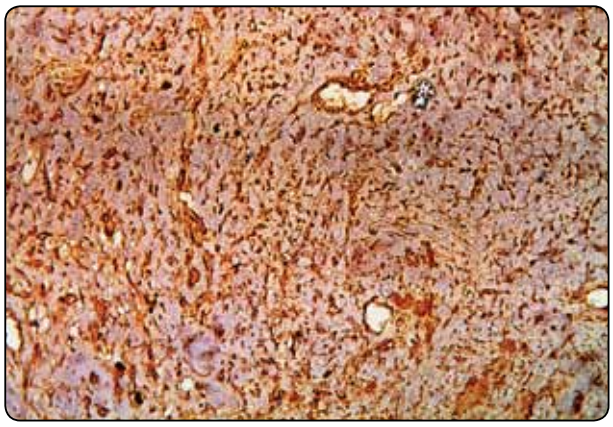

Fig. (17): A photomicrograph of the pulp tissue of deciduous 3rd premolar during shedding showing positive Fas immunoreactivity in all pulp cells (Fas Orig.mag. X200)

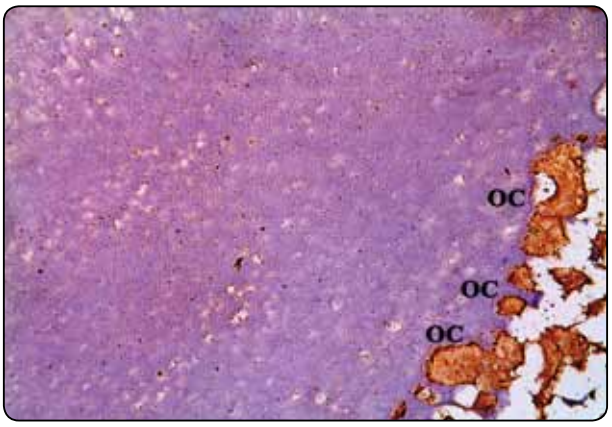

Fig. (18): A photomicrograph of the 3rd premolar during shedding showing positive Fas immunoreactivity in the entrapped odontoclasts (OC) along the resorbed dentine surface (Fas Orig.mag. X 200)

DISCUSSION

Physiological root resorption is a phenomenon that occurs only in the primary dentition. During physiological resorption, all root tissues including cementum, dentin and pulp are eliminated[5]. Cementum and dentin are eliminated by odontoclasts which are specialized in the resorption of these dental tissues. However, the mechanism responsible for the physiological resorption of the pulp tissue hasn't yet been clarified. The permanent tooth follicle and its periodontal ligaments adjacent to primary tooth help in development and activation of the odontoclasts [9-12].

Two essential processes were associated with shedding of the deciduous teeth, root resorption of the primary teeth and eruption of the permanent teeth. Although these two processes occur simultaneously, it was demonstrated that they were not conducted by a single physiological mechanism. Permanent tooth eruption is guided by the periodontal ligaments. However, the way of activation of this eruptive force and the mechanism of the radicular resorption of primary teeth were unclear. Besides the mechanic-compressive forces involved in the permanent tooth eruption, evidence was existed of the occurrence of physiological root resorption of primary teeth in the absence of corresponding permanent teeth [13& 14].

Histological examination of the 3rd deciduous premolars during shedding in dogs revealed resorption of the internal dentin surface in the crown as well as the root with numerous entrapped odontoclasts. This finding was in agreement with previous studies. It was demonstrated that, in human deciduous teeth, odontoclastic resorption activity occurred at the pulpal surface prior to shedding and this internal resorption occasionally extended coronally from the dentinoenamel junction into the enamel [15&16]. There was also a characteristic scalloped line of resorption and entrapped odontoclasts on the internal dentin surface. This line appeared deeper at the apical dentin than the coronal dentin. This finding indicated beginning of resorption along the entire dentin surface at the root as well as the crown but resorption was preceded at a faster rate in the root than the crown. This finding also referred to the time of development of odontoclasts. It indicated that odontoclasts development was early in the crown as well as the apical part of the root. However, it was previously reported that, TRAP-positive mononuclear cells were initially detected in the pulp chamber as root resorption

neared completion. Then these mononuclear cells first made contact with the predentin surface by their elongated cellular processes. After attachment, they spread out along the predentin surface and developed specialized membrane structures, clear zones, and ruffled borders. Finally, they fused with each other on the predentin surface and formed the typical multinucleated odontoclasts [17].

This study revealed that the pulp core showed numerous dilated blood vessels engorged with red blood cells, a lot of large degenerative vacuoles and the pulp tissue at the apical area demonstrated heavy infiltration of chronic inflammatory cells. This finding indicated that, the process of the internal resorption of dentin of deciduous teeth clearly showed histological changes in the pulp in the form of pulp hyperemia coronaly indicating reversible pulp inflammation and chronic inflammation apically. This chronic inflammatory reaction was detected apically in spite of beginning of root resorption. On the contrary, it was reported that during active root resorption, the pulpal tissue retained its normal structure. However, when root resorption neared completion, inflammatory cells started to gradually infiltrate into the pulp [15]. It was also reported that, while the root was resorbed more than one half way, some normal pulp was replaced by the connective tissue as in inflammation and by the time deciduous root resorption was completed and normal pulp tissue was no longer present [18].

Some specimens revealed beginning of coronal dentin resorption under one of the cusps and presence of localized areas of reparative dentin under other cusps. These localized areas of reparative dentin were lined by thin layer of predentin. However the odontoblastic layer was degenerated. This might be due to associated formative and resorptive changes in the pulp of deciduous teeth during shedding. The predentin was also reported to be resorbed by odontoclasts but more resistant to resorption than dentin [19]. Presence of reparative dentin in sound (non carious) deciduous teeth wasn't detected in other previous studies. It might be a unique feature for the deciduous teeth of dogs due to their special mode of feeding on hard materials or as a compensatory mechanism for severe attrition or abrasion. Healing of resorbed roots of deciduous teeth was found to occur by cementum deposition. It was reported that, in the later stage of exfoliation in human deciduous teeth, odontoclastic resorption didn't not continue until the teeth are shed, and the resorbed pulp chamber wall was usually repaired by cementum-like tissue deposition. It might play some role in the retention of deciduous teeth until shedding [20].

Electron microscopic examination of the pulp tissue of dog deciduous teeth during shedding revealed presence of numerous odontoclasts and different types of inflammatory cells including macrophages, monocytes, neutrophils, lymphocytes and plasma cells. Previous studies on shedding revealed that, multinucleated giant cells, referred to as odontoclasts (osteoclasts) were the principal mediators of physiological root resorption of deciduous teeth. Although the resorption organ (resorptive tissue) also contained many fibroblasts, mononuclear phagocytes (macrophages), and granular leucocytes (neutrophils). However, their functional roles in root resorption were not yet known [21-24]. It was also suggested that, macrophages participate in the total process of bone remodeling and tooth resorption. phagocytosis of mineralized bone particles by macrophages had been reported both in vivo and in vitro [25&26]. Further study has demonstrated the presence of N-acetyl-f3-glucosaminidase, which was related to the hydrolysis of glycosaminoglycans, in the macrophages present in periodontal ligament during shedding [27].

Mononuclear osteoclasts and odontoclasts with a ruffled border were also detected in the pulp of human deciduous teeth during shedding. TRAP activity was detected in both multinucleated and mononuclear odontoclasts. But Only 2.9% of odontoclasts were mononucleated and 93.8% were multinucleated. So that, mononuclear odontoclasts were suggested to be participated in human deciduous tooth resorption together with multinucleated ones [28]. It was also suggested that, dental pulp may have cytokine-producing cells which would mediate monocyte-macrophage lineage to form osteo/odontoclasts involved in human primary tooth resorption. Such findings seem to indicate a role of the pulp in the physiological root resorption [8].

Ultrastructure examination also demonstrated presence of two types of fibroblasts. Active formative cells (fibroblasts) surrounded by different sections of collagen fibers and resorptive cells (fibroclasts) surrounded by degenerated collagen fibers. A similar finding was detected in the periodontal ligament of feline deciduous teeth during shedding. Two phenotypically different populations of fibroblasts were detected in the region of ligament resorption: one with numerous collagen-containing phagosomes in the cytoplasm, and the other with condensed nucleus and cytoplasm [22].

Moreover, some mesenchymal cells including fibroblasts, cementoblasts, and mononuclear phagocytes (macrophages) were reported to have a great role in physiological root resorption of feline deciduous teeth. Electron microscopic examination revealed that, in an early phase of root resorption, the resorption organ consisted of many fibroblasts and relatively few macrophages and odontoclasts, the last with a wide, clear zone and narrow, immature, ruffled border. In the active phase of root resorption, the resorption organ contained many odontoclasts with a well-developed ruffled border and a reduced clear zone, cementoblasts, fibroblasts, macrophages, neutrophils, and many blood vessels. In the resting phase of root resorption, the dentin surface was covered mostly with cementoblasts resembling bone lining cells. There was an occasional macrophage, but no odontoclasts were observed during this phase. During removal of the periodontal ligament concomitant with root resorption, many fibroblasts phagocytosed mature collagen fibrils were detected [29].

Electron microscopic examination revealed presence of numerous apoptotic cells with characteristic surface blebbing and distinct apoptotic nuclear morphology with the formation of apoptotic bodies. This finding indicated that apoptosis was involved in pulp cells elimination in physiological root resorption in primary teeth. This result is in accordance with other results as presence of apoptotic cells in the odontoblastic and subodontoblastic layers in rats and human had been described [5]. Histological features of apoptosis were also detected in the pulps of human primary teeth during root resorption. In Haematoxylin and Eosin stained sections, the apoptotic pulp cells appeared shrunken with clear halos surrounding them (anoikia) and characterized by cytoplasm and nuclear condensations. Apoptotic bodies and *in situ* detection of DNA fragmentation by TUNEL reaction were also found [30].

Ultrastructure examination of the pulp during shedding revealed presence of numerous sections of myleinated nerve fibers. This might be an indication of persistence of pulp vitality during shedding. It has been shown that primary teeth retain the potential for sensation, healing, and repair until reaching advanced stages of physiological root resorption [7].

Immunohistochemical results were in accordance with the electron microscopic findings. Immunohistochemical examination of the 3rd

deciduous premolars during shedding revealed intense positive Fas immunoreactivity in all pulp cells (formative cells or defensive cells) indicating apoptosis. So that, both of the electron microscopic and immunohistochemical results of the present study seem to indicate the participation of apoptosis in pulp tissue elimination during physiological resorption of primary teeth. The entrapped odontoclasts along the internal dentin surface also showed positive Fas immunoreactivity indicating their apoptosis. This finding was in accordance with previous study as odontoclast fragments of various sizes suggesting apoptosis were detected during the physiological root resorption in human primary teeth [31]. It was also reported that, after termination of odontoclasts resorptive function, they lost their ruffled borders and became detached from the resorbed surface. Most of the detached odontoclasts had numerous large pale vacuoles and secondary lysosomes and appeared to be degenerated [17]. In conclusion, this study has demonstrated several changes in the pulp of deciduous teeth during shedding in the form of:

1- Hyperemia (reversible pulpitis) in the pulp chamber and chronic inflammation at the apical end regardless beginning of root resorption or after extending of resorption till the mid root.

2- Multinucleated odontoclasts were detected to be entrapped on the internal dentin surface indicating beginning of resorption along the entire internal dentin surface both coronal and apical.

3- Odontoclasts are considered as the main cells responsible for shedding.

4- There are other cells in the pulp having a role in shedding as macrophages and fibroblasts.

5- All the pulp cells (formative or defensive) undergo apoptosis.

6- Well formed myelinated nerves were found indicating pulp vitality and sensitivity during shedding.

7- Odontoclasts undergo apoptosis.

8- Areas of reparative dentin lined by predentin might be detected.

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