The fine structure of the mature odontoblasts and cell rich zone of the human dental pulp

R. Harris and C. J. Griffin
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Introduction

The majority of electron micrographic studies on the odontoblast have been made on cells at the stage of dentinogenesis. Frank described the fine structure of the odontoblast in teeth from subjects 12-15 years of age but no study appears to have been reported on the fine structure of the odontoblast in the pulps of adults’ teeth.

Material and methods

The pulps of normal teeth removed from patients aged 25-35 years were obtained by cutting under saline with a fissure bur in an air rotor and splitting each tooth with bone cutting forceps. The time between removal of the teeth and placing the pulp tissue in fixative did not exceed two minutes in each instance.

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Portions of the coronal pulp were cut into small pieces and fixed in Palade’s (1952) osmium tetroxide fixative at 0°C. for 2 hours—2 per cent osmium tetroxide solution (5.0 mL), veronal-acetate buffer (2.0 mL), distilled water (1.0 mL), and 0.1 N HCl (2.0 mL), to bring the solution to pH 7.3-7.5. Following fixation, the pieces were dehydrated in increasing concentrations of acetone (15 min. in each of 25, 50 and 75 per cent acetone), and, after rinsing in three changes of 100 per cent acetone, embedded in Araldite. Polymerization was effected at 60°C. for 24 hours.

Sections 4-0μ thick were cut, stained and examined by light microscopy; odontoblasts and the cell rich zone were identified in two pulps from the upper canine and upper first premolar. From the blocks which showed these structures, sections 500-1000Å thick (cut on an L.K.B. ultramicrotome and stained with uranyl acetate on the grid for 1 hour) were examined on an Hitachi H.S.7 electron microscope.

Observations

This method of obtaining odontoblasts is essentially by avulsion of the odontoblastic processes from the dentinal tubules and therefore some damage to the cells may occur. However, the cell membranes appeared to be intact and no disrupted cell processes were seen. For this reason apparently only minor trauma has been inflicted on the pulp tissue.

1. Cell body of odontoblasts

The nuclei of odontoblasts in longitudinal and transverse section had a peripheral condensation of chromatin and the outer nuclear
Fig. 1.—Odontoblasts in longitudinal section. N, nucleus; p, cytoplasmic process of the odontoblast; m, mitochondria; l, lipid droplets in vacuoles; k, von Korff's fibres; ecs, extracellular substance. x 7,500.
membrane had attached ribosomes with occasional perinuclear cisternae (Fig. 1-4). The mean diameter of 20 odontoblasts measured across the centre of the nucleus was approximately 4.6 \mu m (S.D. 0.22).

The Golgi apparatus was extensive and usually aligned at the basal region of the nucleus and included smooth walled vesicles similar to those observed in the fibroblast of reticulum and clusters of ribosomes were present (Fig. 3, 4). Many mitochondria were found, generally aggregated about the Golgi apparatus (Fig. 6, 9).

Coiled, regularly beaded filaments 50–70\AA in diameter were present in dilated cisternae of the rough-surfaced endoplasmic reticulum and the cytoplasm contained filaments approximately 60\AA in diameter (Fig. 3, 4, 8).

![Image](image_url)

**Fig. 4.**—Vacuoles containing reticular substance in cell body of odontoblast. N, nucleus; v, vacuole; l, lipid droplets; ly, membrane-bounded body (lysosome); pm, plasma membrane; r, ribosomes. \times 50,000.

**Fig. 5.**—Cell body of odontoblast. N, nucleus; m, mitochondrion; pm, plasma membrane; pinocytotic vesicles (arrow); lipid droplets apparently free in cytoplasm and in vacuoles. \times 25,000.

Lipid droplets were observed within all the odontoblasts and the mean diameter of 67 droplets was found to be 0.51\mu m (S.D. 2099\AA). Most of the lipid droplets were seen in vacuoles which also contained a reticulum of fine beaded filaments, although occasionally a lipid droplet was seen in the cytoplasm (Fig. 3, 4, 5). Occasionally what appeared to be pinocytotic vesicles were seen in the cytoplasm (Fig. 5).

The cell bodies, processes and lateral processes of the odontoblasts were attached to the dental pulp; \(^{17}\) \(^{18}\) other vesicles were present at the plasma membrane and in the extracellular substance (Fig. 3, 8, 9).

Membrane-bounded bodies in the cytoplasm, presumably lysosomes, numerous dilated profiles of the rough-surfaced endoplasmic


**Fig. 2.**—Odontoblasts in transverse and obliquely transverse section. N, nucleus; ecs, extracellular substance; v, vacuole; G, Golgi apparatus. \times 5,400.

**Fig. 3.**—Cell body of the odontoblast. N, nucleus; c, outer nuclear membrane; l, lipid droplets; er, rough-surfaced endoplasmic reticulum; G, Golgi apparatus; c, cilia; ly, membrane-bounded body (lysosomes); d, junctional complex; ecs, extracellular substance; vesicles (arrows). \times 24,500.
Fig. 6.—Cell body of odontoblast and von Korff’s fibres in extracellular substance. N, nucleus;  k, von Korff’s fibres; G, Golgi apparatus; m, mitochondria; pm, plasma membrane; b, axonal swellings; ecs, extracellular substance. × 12,000.

Fig. 7.—Cell process of the odontoblast. N, nucleus; er, rough-surfaced endoplasmic reticulum; pm, plasma membrane; ecs, extracellular substance. Note lipid droplets and intracytoplasmic filaments. × 34,500.
adjoining bodies and processes of other odontoblasts by junctional complexes (Fig. 3, 9). The plasma membrane of the cell body usually had a regular contour; when sectioned transversely it was approximately 75Å thick and appeared to be free of amorphous substance (Fig. 5, 9). The plasma membrane of the cell processes was sometimes extremely irregular and had a scalloped form (Fig. 1).

A structure resembling a cilium with a diameter of 2200Å and length of at least 11000Å containing two axial filaments was seen in one odontoblast. It originated deep in the cell and projected towards the plasma membrane (Fig. 3).

2. Odontoblastic processes

The cytoplasm of the odontoblastic processes contained filaments, a few dilated profiles of the rough-surfaced endoplasmic reticulum and occasional mitochondria (Fig. 1, 7). The mean diameter of sixteen odontoblastic processes was 2.1μ (S.D. 0.54).

3. Inter-odontoblastic substance

Von Korff's spiral fibres with diameters of approximately 0.2μ associated with beaded filaments and some amorphous substance were present and consisted of numbers of irregularly striated fibrils approximately 200Å in diameter (Fig. 1, 6, 9).

Cell processes of odontoblasts and other adjacent cells were seen between the cell bodies of the odontoblasts. Structures similar to axonal swellings described by Harris and Griffin in the "plexus of Raschkow" of the human dental pulp contained small mitochondria and macro- and micro-vesicles and appeared to arise from a constricted axon containing densely clumped neurofilaments (Fig. 6, 8).

Microfibrils approximately 60Å in diameter with beaded elements 120Å wide were lying in the extracellular substance and associated with the plasma membrane (Fig. 9, 10).

Fig. 9.—Cell body of odontoblast. N, nucleus; G, Golgi complex; m, mitochondrion; ecs, extracellular substance; k, von Korff's fibres; f, extracellular beaded microfibrils; junctional complexes (arrow). × 15,000.
4. Cell rich zone

The tissues surrounding the basal surface of the odontoblasts were rich in cells and cell processes and there appeared to be no cell free zone in these specimens. The cells comprising this zone were fibroblasts recognized by the dilated profiles of the roughsurfaced endoplasmic reticulum and undifferentiated mesenchymal cells (Fig. 10). These undifferentiated mesenchymal cells were identified by the absence of profiles of the rough-surfaced endoplasmic reticulum and the presence of mitochondria with regular transverse cristae; free ribosomes were virtually absent from the cytoplasm and perinuclear cisternae were not observed. Some electron-dense membrane-bounded bodies, possibly lysosomes, and invaginations of the plasma membrane suggesting pinocytosis were seen.

The extracellular substance contained fine collagen fibrils about 200Å in diameter and usually a moderately electron-dense amorphous substance was associated with them. An example of an amyelinated nerve fibre associated with the basement membrane of a Schwann cell is shown in Fig. 10. The blood vessels of this zone were of the capillary type with a lumen usually composed of one or two endothelial cells external to which were the basement membrane and sparse subendothelial connective tissue (Fig. 11).

Discussion

The structure of the mature odontoblasts conforms to the pattern of the young cells as described by Frank,60 the main features being numerous mitochondria, vesicles in the cell body and numerous lipid droplets in either the vacuoles or the cytoplasm. The presence of fat in odontoblasts has been demonstrated.
histochemically, and its presence in the mature odontoblast may be evidence of maturation or mobilization of fat arising from trauma. Fat droplets have been observed to enter intestinal cells by a pinocytotic mechanism and to be contained in the cisternae of the Golgi apparatus, the agranular and granular endoplasmic reticulum; in fat cells lipid droplets are always seen to be surrounded by a smooth-surfaced membrane. Fawcett observed in liver cells lipid droplets free in the cytoplasmic matrix and not enclosed by a membrane, and reasoned that the absence of a limiting membrane precluded uptake of chylomicrons by pinocytosis in liver cells. The presence of lipid droplets almost always in vacuoles suggests that in odontoblasts the fat is usually absorbed by a pinocytotic mechanism and then is stored in a vacuole. Subsequently the fat may be found discharged at the plasma membrane, or alternatively, vacuoles containing the droplets may pass out of the cell and disrupt. The former method of secretion has been suggested by Frank and Nalbandian and Frank as an explanation of the presence of vacuoles in the odontoblastic processes; the phenomenon has been termed reverse pinocytosis. This mechanism has been observed in ameloblasts, chondroblasts and mesenchymal cells of the dental papilla.

It is probable that the odontoblasts described here are engaged in secondary or reparative dentine formation. This suggestion is supported by lack of alignment of the cells and absence of a terminal web and a terminal bar apparatus. Nevertheless the cells are attached to each other by numerous junctional complexes. Apart from this, numerous mitochondria and dilated sacs of the ergastoplasm indicate that they are actively engaged in synthesizing collagen precursors. It is apparent that these dilated sacs contain beaded micro-

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fibrils which we have previously implicated\(^{(16)}\) as being a protein polysaccharide complex of the ground substance and which may be responsible for initiating precipitation of collagen. These fine filaments extracellularly can be seen to be associated with von Korff’s fibres and may be responsible for the aldehyde fuchsin positive staining after peracetic acid oxidation of these tissues.\(^{(20)}\) The small vesicles observed at the plasma membrane are essentially similar to the secretory vesicles observed in the dental pulp fibroblast\(^{(18)}\) and the periodontal fibroblast\(^{(21)}\) and may be the means whereby the products synthesized in the ergastoplasm reach the extracellular substance.

A structure which appears to be a cilium was seen in one cell and is similar to the mammalian cell cilia described by Fawcett\(^{(22)}\) and rat odontoblastic cilia observed by Jessen\(^{(23)}\) but differ from the cilia in Schwann cells of the adult rat which have a cortical tube.\(^{(24)}\)

Nerve endings were observed at the basal surface of the odontoblasts and between the cell processes. They were similar to the axonal swellings described in the dental pulp\(^{(20)}\) and contained micro- and macro-vesicles. Frank\(^{(25)}\) has also reported nerve endings between the cell bodies of the odontoblasts and demonstrated the presence of myelinated nerve fibres associated with the odontoblastic processes.

It is generally conceded that the dental pulp is only innervated by delta fibres and vaso-motor fibres.\(^{(26)}\) It is therefore most probable that the nerve endings described herein were derived from myelinated nerve fibres.

The undifferentiated mesenchymal cells of the cell rich zone described herein differ somewhat from those described by Frank\(^{(20)}\) in the dental papilla of human fetuses and new-born cats inasmuch as they were not seen to have an elaborate ergastoplasm. They also differ from young fibroblasts of the dental pulp as described by us\(^{(26)}\) in that the crista mitochondriales of these cells were regular and usually transverse, whereas those of the young fibroblasts were not and there was an absence of grossly dilated perinuclear cisternae.

Innervation of this zone was similar to that described in the peripheral portion of the coronal pulp.\(^{(20)}\) Blood vessels of the capillary type were seen in close proximity to the body of the odontoblasts indicating that this area and the odontoblasts are well vascularized.

**Summary**

1. Electron microscopy of odontoblasts obtained by avulsion from teeth of patients aged 25-35 years showed the mean diameters of the cell body and processes were 4-4\(\mu\) and 2-1\(\mu\).

2. Extensive Golgi complex and dilated profiles of the ergastoplasm suggest cells were engaged in protein synthesis.

3. Numerous fat droplets and mitochondria were present.

4. Myelinated nerve fibres were seen between the odontoblasts.

5. Blood vessels were seen in close proximity to the odontoblasts.

6. The cell rich zone consisted of undifferentiated mesenchymal cells and fibroblasts.

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The ultrastructure of small blood vessels of the normal human dental pulp

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Introduction

The ultrastructure of mammalian arterioles and capillary sphincters was studied by Rhodin[10] who described arterioles of approximately 50μ diameter. He noted that terminal arterioles, arising from the arterioles at right angles, gave rise to precapillary sphincters which had diameters of approximately 10μ.

Gavin and Trotter[11] reported observations on the capillaries of the gingival tissue. There does not appear to have been any detailed report on the fine structure of dental pulp blood vessels except for that by Svejda.[12]

In this paper we have classified the arteriolar peripheral blood vessels according to Rhodin.[10] He classified arterioles as vessels having a diameter from 50-100μ and a tunica media approximately 5μ thick consisting of several layers of smooth muscle cells, and terminal arterioles as those vessels having a diameter of up to 50μ and a tunica media with one layer of smooth muscle cells which was approximately 0-5μ thick.

We have used the classification, for capillary blood vessels, developed by Bennett, Luft and Hampton[13] and Simon.[14] The former divided capillaries into two types:

Type A.: Having a continuous basal lamina;

Type B.: Without a continuous basal lamina and with or without the presence of any type of fenestration.

The latter describes three types of capillaries:
1. those without fenestrations and pericytes and with either cuboidal or squamous endothelial cells;
2. Fenestrated capillaries without pericytes;
3. Capillaries with a discontinuous basal lamina.

Materials and methods

The pulps of four normal non-carious upper premolars were extracted from patients aged 15 years. The pulps were obtained using the method previously described by Harris and Griffin,[15] viz.:

Immediately after the teeth had been extracted the crowns were cut under saline with an air rotor and split open with bone cutting forceps. The time between removal of the teeth and placing the pulp tissue in fixative never exceeded 2 min.

Portions of the coronal pulp were cut into small pieces and fixed in Palade's[16] osmium tetroxide fixative at 0°C for 2 hr [2 per cent osmium tetroxide solution (5-0 ml), veronal-acetate buffer (2-0 ml), distilled water (1-0 ml), and 9:1 NHCl (2-0 ml), to bring the solution to pH 7.3 – 7.5]. Following fixation, the pieces were dehydrated in increasing concentrations of acetone (15 min. in each of 25, 50, and 75 per cent acetone) and, after rinsing in three changes of 100 per cent acetone, embedded in Araldite. Polymerization was effected at 30°C for 24 hr. Sections 500-1000Å thick cut in an LKB ultramicrotome and stained with uranyl acetate on the grid for 1 hr. were examined in a Hitachi H.S.3 electron microscope.

Observations

Capillaries

True capillaries approximately 3-7μ in diameter were seen (Fig. 1). They consisted of one to three endothelial cells with marginal folds overlapping at their junctions.

The lumina contained a diffusely granular material; occasionally erythrocytes were present (Fig. 1).

The basal lamina of the endothelial cells usually consisted of three zones, an internal less electron-dense zone, the lamina rara, an intermediate electron-dense zone, the lamina densa, and an external irregular zone, the lamina diffusa. The basal lamina varied in thickness from 130-1500Å (Fig. 1, 2 and 3). In all blood vessels examined a continuous

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basal lamina was present. Aggregations of vesicles with an electron-dense amorphous outer coat were present on the tissue and luminal side of the endothelial cells. These vesicles were of two types, micropinocytotic of 600-700Å diameter, and larger vesicles approximately 1500Å in diameter. Mitochondria with regular cristae, profiles of the rough-surfaced endoplasmic reticulum and a few intracytoplasmic filaments were present (Fig. 1). Fenestrations occurred in the endothelial plasma membrane in 2 of 50 capillaries examined. These fenestrations contained a fine membrane (Fig. 2). Pericytes were not observed in these instances. The endothelial cells were of the squamous variety.

Pericytes partially encircled the other capillary blood vessels. The plasma membrane of the pericytes in juxtaposition to the basal lamina of the endothelial cells appeared to be devoid of electron-dense amorphous material. However, on the tissue side of the cell the basal lamina was apparent. The Golgi complex was juxtanuclear and a few profiles of the rough-surfaced endoplasmic reticulum were seen. Electron-dense bodies, presumably lysosomes, and occasional intracytoplasmic filaments were present (Fig. 3).

Perivascular connective tissue consisted mainly of aggregations of collagen fibrils, in most cases orientated longitudinally (Fig. 3) to the long axes of the vessels, but in other instances orientated both longitudinally and circumferentially to the blood vessels (Fig. 1).

The amount of perivascular connective tissue differed with individual capillaries. In some it was very sparse subendothelial connective tissue (Fig. 1 inset and 2), whilst in others it was particularly dense (Fig. 1 and 3).

Small arterioles

Arteries were seen which consisted of three well-defined coats—the tunica intima, the tunica media and the tunica adventitia (Fig. 4).

The tunica intima consisted of squamous or cuboidal endothelial cells, basal lamina and closely associated fragmented internal elastic lamina (Fig. 4 and 5).

The tunica media, approximately 6μ in width, consisted of two to three layers of smooth muscle cells orientated in a circumferential spiral (Fig. 4 and 6) around the lumen of the blood vessel. The smooth muscle cells of the tunica media had localized condensations (bar-like structures) of cytoplasm next to the plasma membrane (Fig. 4 and 7). Occasionally junctions between neighbouring smooth muscle cells (Fig. 4) and myoendothelial junctions were observed (Fig. 5). The nucleus was somewhat notched with the Golgi complex in close proximity and aggregations of intracytoplasmic myofibrils were responsible for the electron-dense appearance of the cytoplasm. Dilated profiles of the rough-surfaced endoplasmic reticulum and vesicles in association with the plasma membrane were seen. The cells were surrounded by a basal lamina and fine collagen fibrils and elastic fibres were present between the cells (Fig. 7). There was no marked overlapping of the endothelial cells.

The tunica adventitia consisted of collagen fibrils orientated longitudinally and obliquely. Myelinated and unmyelinated nerve fibres were seen throughout the adventitia.

Terminal arterioles

Terminal arterioles were identified as blood vessels with diameters of 10-15μ. The tunica intima consisted of squamous endothelial cells and basal lamina. Vesicles with electron-dense amorphous outer coats 150Å in diameter were observed but they were not as numerous as in the endothelial cells of the capillaries. No fenestrations were observed in the plasma membrane of the endothelial cells. The basal lamina of the endothelial cells and of the smooth muscle cells was continuous (Fig. 8).

The tunica media consisted of one layer of smooth muscle cells (Fig. 8). The tunica adventitia was quite inconspicuous and consisted of small aggregations of collagen fibrils (Fig. 8).

Arteriovenous anastomosis

A blood vessel which we have identified as an arteriovenous anastomosis was characterized by cell bodies of cuboidal endothelial cells projecting into the lumen of the vessels producing a corrugated lumen (Fig. 9). Endothelial cells and smooth muscle cells were separated by basal lamina. The tunica media consisted of smooth muscle cells orientated circumferentially and obliquely. Thick elastic membranes were noted between the smooth muscle cells. The tunica adventitia appeared to consist almost entirely of thick elastic membrane.

Discussion

Capillaries

Four per cent of the capillaries of the dental pulp were found to be fenestrated and these findings are similar to those reported by Gavin and Trotter in human gingival capillaries.
Fig. 1.—Capillaries from human dental pulp. E, endothelial cell; J, endothelial cell junction; B, basal lamina of endothelial cell; Bp, basal lamina of pericyte; P and arrows, microphinocytotic vesicles; Pp, pinocytotic vesicles; A, unmyelinated axon; C, collagen fibrils. × 15,600
N.B.: There does not appear to be any basal lamina on the endothelial side of the pericyte.
Inset: Capillary in cross section. E, erythrocyte. × 9000.

Fig. 2.—Capillary. Ep, endothelial pores seen in inset; Ba, lamina rara; Bc, lamina densa; Bd, lamina diffusa. × 31,000. Inset × 80,000.

Fig. 3.—Capillary. N, nucleus of pericyte; P, pericyte; E, endothelial cell; B, basal lamina of endothelial cell; Bp, basal lamina of tissue side of pericyte. × 16,500.
Note the thick layer of longitudinally orientated collagen fibrils.
Fig. 4.—Transverse section through wall of an artery of the human dental pulp. Tl, tunica intima; Tm, tunica media; Ta, tunica adventitia; S, smooth muscle cells; marginal condensation (arrows); A, axons and Schwann cell cytoplasm, × 9,000. J, junction between two muscle cells. Inset × 25,000.

Fig. 5.—Artery. E, endothelial cell; El, fragmented internal elastic lamina; S, smooth muscle cell; Mj, myoendothelial cell junction. × 25,000.
These fenestrated capillaries did not have associated pericytes. However, other capillaries were observed that did. In all cases the basal lamina was continuous. On this basis dental pulp capillaries can be classified into the two groups:

1. fenestrated capillaries without pericytes;
2. non-fenestrated capillaries.

Seong and Avery\(^\text{10}\) in a study of the hamster's dental pulp reported finding only Simon's type I capillaries.

We observed numerous vesicles in the cytoplasmin of the endothelial cells. They were identified as pinocytotic vesicles because of the characteristic features of their walls, especially the outer electron-dense amorphous coat (Fig. 1). They are said to arise from the luminal and/or basal plasma membrane by the pinching of small inpocketings (Seong and Avery\(^\text{10}\)). Palade\(^\text{9}\) and Farquhar, Wissig and Palade\(^\text{9}\) had noted similar vesicles which transport colloidal particles across the capillary endothelium. It is probable that the vesicles seen by us in the endothelial cytoplasm perform a similar role and therefore are engaged in microinocytosis. Larger vesicles seen in the endothelial cytoplasm may be similarly engaged. It has been suggested that these large vesicles might be formed either by the fusion of the luminal cytoplasm flanges at endothelial junctions (Fawcett and Wittenberg\(^\text{12}\)) or by coalescence of microinocytotic vesicles.\(^\text{12}\)

The basal lamina completely surrounded the tissue side of the endothelial cells. The origin of the basal lamina is uncertain. It has been suggested by Rhodin\(^\text{4}\) that it is formed by pericytes, endothelial cells and smooth muscle cells.

Apparently the basal lamina is an integral part of the endothelium of dental pulp capillaries and Rhodin\(^\text{9}\) states it serves to distinguish blood capillaries from lymphatic capillaries which are without a basal lamina.

The pericytes of other capillary vessels in the dental pulp (Fig. 3) can be contrasted with the smooth muscle cell (Fig. 6). The cytoplasm of the pericyte has markedly less fibrils and attachment sites at the plasma membrane were not seen. Maeda\(^\text{13}\) studied the retina and Maynard, Schultz and Pease\(^\text{14}\) studied cerebral capillaries and noted that pericytes resembled smooth muscle cells. A distinguishing feature was that the cytoplasm of the pericytes contained fewer fibrils than that of the smooth muscle cells and that attachment zones at the plasma membrane were less prominent. Another distinguishing feature was the absence of a basal lamina on the endothelial cell side of the pericyte. All capillaries examined had a continuous endothelial basal lamina. Hogan and Feeley\(^\text{15}\) described the fine structure of small blood vessels in the human, the monkey and the rat retina and referred to their continuous endothelium and basal lamina. They noted the presence of numerous pericytes and their resemblance to smooth muscle cells.

Small arteries and arterioles

The fine structure of the small arteries of the dental pulp corresponded in almost every respect with those described by Rhodin\(^\text{4}\) in the circulatory bed of the rabbit medially thigh muscle. That author described arterioles, precapillary sphincters and capillaries as comprising the vascular elements of the circulation. However, in the pulp we did not observe vessels at right angles to the arterioles and therefore precapillary sphincters could not be identified.

The disposition of the smooth muscle cells in the tunica of the small arteries of the human dental pulp (Fig. 4 and 6) suggest that they tended to have a spiral arrangement. Rhodin\(^\text{4}\) noted a spiral arrangement of the smooth muscle cells of the small arteries of the rabbit's thigh. The fact that muscle cells are joined not only to themselves but also to endothelial cells by tight junctions indicates the existence of a more elaborate contractile mechanism than present in larger arterial blood vessels which do not have myoendothelial junctions.

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Fig. 6.—Artery of human dental pulp. Lu, lumen; E, endothelial cell; S, longitudinal section, transverse section of smooth muscle cells. × 6,750.

Fig. 7.—Smooth muscle cell. N, nucleus; L, lysosome; G, Golgi complex; M, marginal condensation of cytoplasm; B, basal lamina; El, elastic membrane; P, pinocytotic vesicles; C, aggregations of collagen fibrils to form reticular fibres. × 29,500.

Fig. 8.—Composite picture of terminal arteriole. Ne, nucleus of endothelial cell; S, smooth muscle cell; myoendothelial junction (arrows). × 4,500. Inset shows tight junction of membranes between endothelial cells. Inset × 67,500.

Fig. 9.—Blood vessel in transverse section. El, elastic membrane; S, smooth muscle cell oriented circularly; Sc, smooth muscle cell oriented longitudinally; Ne, nucleus of endothelial cell; Na, nucleus of smooth muscle cell. This blood vessel has been tentatively identified as an arteriovenous anastomosis. ×12,000.
This mechanism indicates that when the smooth muscles of these arterioles contract not only do they shorten themselves but because of their endothelial cell connections they exert a direct effect on these cells.

**Peripheral circulation**

The essential features of the coronal pulp circulation as observed by us were the presence of small arteries-arterioles-capillaries and arteriovenous anastomoses. The latter structures were identified by the presence of a corrugated lumen and smooth muscle cells of the tunica media without any special orientation (Fig. 9). Provenza\(^{[20]}\) described the peripheral circulation as containing arteriovenous anastomoses connecting an arteriole to a vein and stated that the tunica adventitia was virtually non-existent. Arteriovenous anastomoses have been described by Popoff\(^{[20]}\), Brown\(^{[20]}\), Dawes and Prichard\(^{[20]}\) and Clara\(^{[20]}\) as being characterized by a corrugated lumen, absence of an internal elastic lamina, a tunica media consisting of smooth muscle cells randomly orientated, epithelioid cells, and an adventitia composed of a neurocollagenous reticulum.

As stated above, we were unable to identify precapillary sphincters. The peripheral circulation of blood in the human dental pulp is described by light microscopy\(^{[20]}\)\(^{[20]}\)\(^{[20]}\) as follows—from the arteriole and metarteriole precapillary sphincters lead to the thoroughfare channel which communicates with an efferent vein. True capillaries arise from the thoroughfare channel and drain back into it. This description corresponds to the description of the peripheral circulatory system given by Zweifach.\(^{[20]}\) Usually before the capillary circulation is established an arteriovenous anastomosis connects the afferent arteriole to the efferent vein. Because of this, alternate circulatory routes are available. In one instance with the vessels at rest blood passes from the arteriole to the vein via the relaxed arteriovenous anastomosis and very little passes through the tissues. During function the arteriovenous anastomosis shuts down, precapillary sphincters relax and the blood is transported to the tissues via the thoroughfare and true capillaries. The thoroughfare channel is usually identified under light microscopy by the thickness of the perivascular connective tissue. It is interesting in this respect to contrast the perivascular connective tissue of the capillaries demonstrated in Fig. 1 (inset) and Fig. 2 with the perivascular connective tissue shown in Fig. 1 and 3 in which the latter is much thicker. This suggests that the vessels shown in Fig. 1 and 3 may be thoroughfare channels.

**Summary**

Electron micrographs of tissue from human dental pulps of four teeth showed the structure of capillaries, arteries and terminal arterioles. Penetrations were present in 4 per cent of the endothelial plasma membrane of capillaries and the amount of perivascular tissue varied.

Two and sometimes three layers of smooth muscle cells in small arteries and only one layer in terminal arterioles were observed. Myelinated and unmyelinated nerve fibres were seen in the tunica adventitia of larger vessels and one vessel was tentatively diagnosed as an arteriovenous anastomosis.

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The healing of the traumatized dental pulp following capping

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with the technical assistance of Christa Lossin
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Introduction
The maintenance of the integrity of the dental pulps is surely the paramount objective of the dental operator. Despite the need to satisfy a number of other demands in the restoration of defective units in the dental arches, the importance of a healthy pulp cannot be denied.

It is therefore unfortunate that, when disease or trauma breaches the pulpal integrity, a series of events may follow which may end in the loss of the affected tooth. During the course of these events the patient may be exposed to risks which in some circumstances, such as bacterial endocarditis, may terminate in serious disability.

The pulp, as with other connective tissue, when subjected to trauma of any kind is affected by the influences of the various phenomena associated with inflammation. For inflammation is a reparative process and in its early stages the reactions of vascular permeability and pain are present. However, because of the special relation of the pulp to its dense unyielding container and, in the case of the adult somewhat restricted blood supply, repair is not a straightforward process. In attempts to facilitate repair, pulp-capping for a long time has been the selected treatment. As a successful procedure its popularity has waxed and waned. Currently, with the introduction of antibiotics and certain of the corticosteroid preparations, an increase in interest is apparent.

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Grossman(1) says “it is not recommended for adults’ teeth because resistance of the pulp is low and repair is uncertain”. He has further indicated that repair may be expected only from simple exposure of a healthy uninfected pulp (op. cit.). As Dorfman, Stephan and Muntz(2) have shown the deepest layer of softened dentine is sterile in most cases. The placing of an inert base over such dentine could be followed by reparative reaction from the pulp or an exposure of the pulp could be capped satisfactorily, if the operative procedures have been performed under sterile conditions with rubber dam. Glass and Zander(3) have observed that differences occur in the reaction of the pulp to treatment. They found that, when zinc oxide eugenol paste was used, healing was not observed; the pulp remained vital but chronic inflammation persisted at the site of trauma. However, when calcium hydroxide was used over the exposed pulp, rapid healing relatively free of inflammation was seen. “Within four weeks the original site of the exposure is completely walled off by a new odontoblastic layer and a new dentine barrier”. The patients treated by Glass and Zander were aged 9-15 years and both agents were tested in each subject.

Unfortunately most pulps are damaged during removal of carious dentine or by the carious process so that in fact the pulp has already been injured before it is exposed.

Furthermore, it is extremely difficult with the methods currently available to find a correlation between the histologic examination and the clinical status of any tooth.\(^{(4)}\)

The obvious advantages of successful pulp-capping are several but, when used for a pulp of a partially developed tooth are most important since, if it is successful, the pulp is protected and thus enabled to proceed with the normal development of the root. Usually after successful pulp-capping the tooth should be without symptoms or be only slightly hypersensitive to thermal changes for a brief period immediately after treatment.

However, because of our inability to correlate clinical assessment and histological findings, success may be apparent only. Reaction to thermal changes leading to painful symptoms and subsequent pulpal degeneration may follow in what was formerly a symptomless tooth. The action of calcium hydroxide is one of superficial necrosis beneath which the cells undergo a metaplasia and an active odontoblast-like layer arises upon which is formed secondary or repair dentine.

An extension of the technique is the operation of pulpotomy in which the coronal portion of an uninfected vital pulp is removed and calcium hydroxide applied to the non-bleeding, cut surface of the pulp. If successful, a complete dentine bridge develops across the region of operation. In attempts to widen the effectiveness of the procedure antibiotics have been added to the calcium hydroxide but its high alkalinity (pH 12) destroys penicillin and chloromycetin.\(^{(5)}\) The most recent introduction has been that of corticosteroids.\(^{(6)}\) Their use has been supported on the basis of their role in inflammation. However, although these hormones suppress inflammatory reaction, they do not inhibit the growth and spread of bacteria; consequently antibiotics have been incorporated.\(^{(6)}\) After such treatment Rapoport and Abramson\(^{(6)}\) found a quite high percentage of teeth remaining vital and classified the treatment as successful when the teeth responded to electrical stimuli in the same manner as vital teeth.

Kiryati\(^{(7)}\) made observations on the pulps of rat molars using corticosteroid alone and in combination with polyantibiotics. The pulps were damaged and infected before treatment. When oxytetracycline and chloramphenicol together with the hormone were used, 63 per cent of the pulps showed complete healing compared with only 22 per cent with the use of the hydrocortisone alone. The addition of calcium carbonate or calcium hydroxide did not interfere with the repair process and in fact the latter increased the amount of calcific repair.

Schroeder and Triadan\(^{(8)}\) found that pain was controlled quickly in pulps which had been exposed and then treated with a paste made of the glucocorticosteroid triamcinolone, chloramphenicol and xylocaine. The teeth were subsequently capped with zinc oxide eugenol plus one third weight of triamcinolone. Baume\(^{(9)}\) extended the study further and incorporated calcium hydroxide in a mixture similar to that of Schroeder and Triadan and found that there was a definite calcific barrier formed in the pulps of human teeth, and furthermore that it appeared essential for calcium hydroxide to be incorporated for this to occur.

The advent of the corticosteroid has led to a resurgence of interest in pharmacotherapy for injuries to the pulp and the Introduction to Australia of materials based on their use is of sufficient importance to warrant an investigation of reactions. This preliminary report presents some of the findings in permanent teeth of children. It in no sense should be considered a definitive study but is an indication of the reactions that have been observed. For comparative purposes some mention will also be made of the results from applying similar methods to the pulps of the upper first molars and lower incisors of white rats.


Materials and methods

1. Capping compounds
(i) Glucocorticosteroid*

(a) Powder:
Triamcinolone acetonide 0-67 per cent
Demethylchlorotetracycline
Calcium 2-00 per cent
Zinc oxide
Calcium hydroxide

(b) Liquid:
Eugenol
Turpentine oil.

(ii) Calcium hydroxide in a methylcellulose base.

2. Bacteriological test
The bactericidal efficiency was determined of various materials used in pulp-capping procedures, including a number of proprietary calcium hydroxide compounds against inocula of the following organisms: yeast, Staphylococcus aureus, α- and β-streptococci, Streptococcus salivarius, Neisseria catarrhalis and lactobacilli.

3. Teeth
(i) Rat: Forty-four teeth (21 calcium hydroxide, 23 glucocorticosteroid) were treated and the time of recovery spread over 2 to 22 days. In addition three pairs of lower incisors were studied. In one pair zinc oxide eugenol paste, and in the other two a paraformaldehyde compound was placed through an opening in the root via a surgical flap. The cavities were sealed with amalgam.

(ii) Human: Teeth indicated for exodontia as part of orthodontic therapy were used in patients willing to cooperate in the study. All teeth were subjected to thermal tests for an assessment of pulp reaction and only teeth judged as vital were used in the study. After exposure of the pulp under a rubber dam and cessation of hemorrhage, the appropriate agent was placed directly on the pulp tissue, covered with zinc oxide and eugenol and the cavity sealed. Each patient returned within seven days and reported the reaction.

Fifteen teeth have been recovered of which five were treated with calcium hydroxide and nine with glucocorticosteroid. The time-interval ranged from 38 to 145 days for calcium hydroxide and 28 to 314 days for glucocorticosteroid. One tooth with zinc oxide eugenol paste for 87 days was used as a control.

### Table 1

<table>
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<th>Day of recovery of tooth</th>
<th>Calcium hydroxide</th>
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### Results

1. Rats' teeth
Forty-four teeth were subjected to pulp exposure by round bur and pulp-capping with calcium hydroxide (21) and glucocorticosteroid (23). In Table 1 the repair reaction to either compound is listed. It will be noted that repair reaction was taking place in 10 teeth with

* *Ledermix*, a product of Cyanamid International, Lederle Division.

![Fig. 1.—Pulp exposed in upper first molar of rat (35B). Glucocorticosteroid sealed in place for 6 days. Note calcific barrier around site of exposure. Haematoxylin and eosin. X 50.](image-url)
Fig. 2.—(a) Pulp exposed in upper first molar of rat (24A). Glucocorticosteroid sealed in place. On recovery of specimen amalgam restoration found to be missing. Note inflammatory zone beneath the region of repair barrier. (b) Odontoblast zone maintained and large blood vessels in radicular portion of pulp. Hematoxylin and eosin. × 60.

calcium hydroxide and 15 teeth with glucocorticosteroid. In the case of the latter, signs of repair were present as early as four days after treatment and one tooth showed no signs of repair even after 22 days. Figure 1 is an example of a repair barrier developing in a satisfactory manner, whilst Fig. 2 shows some inflammatory reaction beneath the reparative zone where the amalgam restoration had been lost. The radicular portion of the pulp in all cases showed little or no signs of reaction. A good example of repair barrier is shown in Fig. 3 and may be compared with normal dentinogenesis shown in Fig. 4.

In the lower incisor teeth where the zinc oxide and eugenol and paraformaldehyde paste were placed through surgical exposure in the bone, evidence of calcific repair and the development of an odontoblast-like layer was well established at the twenty-second day.

2. Human teeth

Repair calcification was present in three of the five teeth treated with calcium hydroxide, and in the one with zinc oxide eugenol paste.

Fig. 3.—Upper right molar of rat (35RA): pulp exposed and calcium hydroxide placed over pulp and cavity sealed with amalgam for six days. Note well defined repair barrier and cellular arrangement. P.A.S. × 250.

Fig. 4.—Normal odontoblast zone with predentine, upper molar of rat (27BE). Hematoxylin and eosin. × 600.
in some of the teeth treated with calcium hydroxide as shown in Fig. 5. However, some zones of amorphous calcific material were found beneath the region of the pulp exposure in six teeth. These amorphous calcific areas did not completely bridge across the pulp tissue. It is suggested that they indicate a disturbance of calcific reaction or of dentinogenesis.

In all cases treated with glucocorticosteroid the patients reported cessation of pain and no further symptoms in the tooth.

One painful tooth had had a carious cavity filled with zinc oxide eugenol paste for 80 days. In spite of removal of the dressing and all carious dentine under local anaesthesia and capping of the exposed pulp with calcium hydroxide there was constant pain for seven days. The calcium hydroxide was removed and glucocorticosteroid cement base placed in the cavity and sealed. Relief of pain followed immediately and the tooth remained symptomless: Ten days later it was extracted.

Evidence of some inflammatory reaction was present in seven teeth treated with glucocorticosteroid but this was not as extensive as in three of the teeth treated with calcium hydroxide (Fig. 7). It is also of importance to note that in two teeth treated with calcium hydroxide and seven treated with glucocorticosteroid, calcific deposits were present.

In two of the teeth treated with calcium hydroxide intrabulbar calcification was found on the walls of the root canals.

In eight of the teeth treated with glucocorticosteroid similar calcific reaction was found in part of the walls of the root canals and on the floor of the pulp chamber.

At the site of the pulp exposure there was no evidence of dentine bridging such as seen
on the walls of the root canal. These deposits may be manifested in a broad area along the canal (Fig. 8) or consist of a series of spherical outgrowths into the soft tissue (Fig. 9). In addition, amorphous, calcified areas, darkly staining with hematoxylin and eosin, were seen in the coronal pulp occasionally and in the radicular pulp frequently (Fig. 10a, b). A common finding in all teeth treated with glucocorticosteroids was the presence of large and in some cases numerous venous sinuses devoid of blood cells (Fig. 11, 12). The absence of blood cells in these large sinuses may be the result of artefacts during processing, since small branches of these cavities do contain them (Fig. 12c.). In general, the effect of glucocorticosteroids would appear to be a reduction in the area of the pulp involved rather than the elimination of inflammatory reaction.

Attempts by the pulp to lay down a calcific repair barrier completely sealing the dentine defect were not demonstrated, although in one tooth treated with calcium hydroxide this had almost occurred in 145 days (Fig. 5). It should also be noted that in this example the cellular
tissues of the pulp appeared to be functioning normally. Where glucocorticosteroid was used on an exposed pulp (Fig. 6) calcification was of an amorphous type with an absence of odontoblast-like cells (compare with Fig. 5).

3. Bacteriological test

The agents effectively inhibiting growth at 24 hours with all organisms were: glucocorticosteroid cement, glucocorticosteroid liquid, zinc oxide and eugenol, paraformaldehyde solution. Calcium hydroxide alone was ineffective or weakly inhibiting in most cases and reduced the effectiveness of penicillin when the latter was combined with it; whilst the glucocorticosteroid paste was ineffective against yeast and weakly effective against \( \beta \)-streptococci.

Discussion

It is emphasized that this report can only be accepted as a preliminary study of an important field of pulp therapy. Such investigations, to have any value, must be carried out on the human dental pulp.

One of the major difficulties is the lack of precise information on the status of the pulp either before, during or after treatment. Attempts have been made to classify the status on the results of various electrical and thermal tests. Importance has been placed on the severity of pain either as a symptom or arising from certain testing stimuli. However, histological evidence has shown that the severity of pain is only partially related to the degree of inflammatory response. A recent and carefully correlated study by Seltzer, Bender and Zlontz\(^{10}\) has shown that much of the older classification of hyperemia, partial pulpitis, and necrosis, cannot be clearly defined in


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Fig. 9.—192/1: Carious exposure in upper left first molar (same patient as for Fig. 8). Glucocorticosteroid placed in floor of cavity and sealed for 144 days. Note atubular dentine on floor of pulp chamber and walls of canal. Hematoxylin and eosin. x 60.

Fig. 10.—177/1: (a) Caries free lower right first molar in child aged 15 years. Pulp exposure made under rubber dam. Calcium hydroxide "capping" in place 94 days. Dentine debris in pulp forming a nidus for calcification and area of necrosis. No definite barrier of calcific repair beneath exposure. Hematoxylin and eosin. x 15. (b) Root canal with areas of calcific deposits throughout pulp. Hematoxylin and eosin. x 15.
relation to clinical observations. The severity of the painful response is only partially related to the severity of the inflammatory response. It has been shown that acute inflammatory reaction can be demonstrated in the pulps of teeth subjected to operative procedures without the production of severe pain, and we are all familiar, of course, with extremely painful responses in teeth where operative procedures have not been carried out with care or with the precaution of restricting rise in temperature 

![Image](image_url)

Fig. 11.—191/1: Carious exposure on mesial cornu of upper right molar in child aged 12 years. Glucocorticosteroid placed over exposure and tooth removed after 23 days. Note absence of calcific barrier, inflammatory reaction slight and large venous cavities. Haematoxylin and eosin. x 60.

during cutting. One factor which influences the presence of pain is that of drainage. For example, should during cavity preparation an exposure of the pulp be made and drainage of inflammatory exudate follow, pain may be absent, unless the actual site of trauma is directly stimulated. On the other hand, we are all aware clinically of those teeth which provide an excited response to even a slight stimulus so that it seems that the severity of the pain may be quite unrelated to the type of stimulus applied to the tooth whether it be heat or cold, electrical response, or sweet or sour substances. Further, pain responses to specific stimuli are not pathognomonic and inflamed pulps have been shown to react to either heat or cold stimuli. In general, pain is usually associated with exposure of the pulp. Pain following the insertion of a restoration in a deep cavity may be the result of the superimposition of an acute inflammation on an existing chronic one, since it has been shown that acute inflammation from operative procedures rarely produces severe pain.

One of the diagnostic tests which is usually quite positive is that of pain on percussion. This indicates that the reaction has proceeded to the stage involving the periodontal membrane, but this progress may follow even when total inflammatory response by the pulp has not occurred. It was usually assumed that in a state of chronic inflammation a rise or fall in temperature was sufficient stimulus to produce a painful response. Here again histological evidence has shown that a response may occur from one or other or both of these stimuli or a response may be absent despite the presence of inflammation.

Chronic pulpitis, which may arise from operative procedures may not produce symptoms and subsequent treatment may produce a situation in which an acute inflammation is superimposed and then the patient reports a severe exacerbation of pain. Despite the numbers of papers that have been presented in attempts to correlate the findings of pulp testers and pulp testing methods using electrical stimuli or heat and cold, there has been only a poor correlation established between the results of these tests and the histological status of the pulp, except in the case of the necrotic pulp in which the electrical tests are the most accurate. Since the reaction in the pulp is similar to inflammatory reactions elsewhere, it is possible to have areas of pulp in which necrosis has occurred while the remaining portion remains viable. In these cases pulp testing may indicate complete necrosis.

Contrary to popular belief, the amount of reparative dentine which forms on the walls of the pulp chamber subsequent to some traumatic incident does not necessarily result in delayed responses to various tests. It is also a clinical experience that apical rarefaction may be demonstrated by radiographical examination in chronic pulpitis but nevertheless the apical portion of the pulp may remain viable, which phenomenon may be simply due to the existence of auxiliary canals.

A common classification of pulp status is that of hyperaemia and it is said that a tooth in which there is a hyperaemic pulp is more sensitive to various stimuli than a normal tooth. The condition is supposed to be one in
which blood vessels are dilated. However, dilated blood vessels are present in so-called normal pulps and may be found in atrophic pulps as well as in those in which an acute inflammation exists. It should not be overlooked that the vessels may have been dilated during the extraction of the tooth. In general it should be reiterated that operative procedures may produce inflammatory reactions which are transitory. Contrary to most statements, inflammation of the pulp is not irreversible. It should be remembered that, where caries exists, the pulp may already be chronically inflamed so that subsequent operative procedures convert this chronic inflammation to one of acute inflammation, in which case one of two things may happen. The pulp may still recover or it may revert to a chronically inflamed state for a long period of time apparently without many symptoms.

From what has been said, it is apparent that the procedure of capping a pulp which has been exposed to carious processes may have a doubtful prognosis, because it may already have large areas of chronic inflammation in which liquefaction necrosis has occurred and such treatment would prevent the establishment of drainage. Such pulps should receive endodontic therapy, otherwise a pulp with chronic inflammation may become entirely necrotic without pain. Ultimately liquefaction

Fig. 12.—578/1: (a) and (b) Instrumental exposure of pulp in upper right molar in child aged 14 years. Glucocorticosteroid sealed over pulp for 57 days. Note absence of inflammatory reaction but many large venous cavities. Some tubular calcification forming around debris. x 60. (c) Area "X" showing in (b). Haematoxylin and eosin. x 250.
necrosis may occur in the apical granulation tissue and acute periodontitis follow. Where an acute suppurative inflammation has developed, it usually results from some operative procedure after which liquefaction necrosis has occurred in portion of the pulp. The presence of pus causes the painful response.

It will be seen therefore that the prognosis for treatment to the pulp depends upon the actual state of the pulp at the time. Since it is extremely difficult to define this precisely, the results may be all too readily attributed to the treatment yet may have existed prior to it. Langeland\(^{15}\) has shown that inflammatory cells may be demonstrated in the pulp even in the absence of caries. Mitchell and Tarplees\(^{16}\) in a study of 26 teeth with caries, found three that were symptomless in which pulpsitis was confirmed on histological examination; all 26 were sensitive to cold and 25 to heat, but the 22 sensitive to percussion had no inflammatory reaction in the apical third. All teeth had a partial pulpsitis involving either the cornua, or extending through the coronal pulp even to the entrance of the root canals. Some teeth with only a pulp cornu exposed and a limited zone of inflammation were sensitive to percussion.

Dachi\(^{17}\) found that hyperemia can be significantly associated with increased sensitivity to both heat and cold. He also noted in his material (caries-free teeth in which Class V restorations were inserted seven days prior to extraction) that very few teeth were free from inflammation and that increasing degrees of inflammation were associated with a significant increase in sensitivity to heat.

More recently, Seltzer, Bender and Nazimov\(^{18}\) state, “All clinical tests are diagnostic aids, but no single one of them can be conclusively or exclusively used for a definite diagnosis. . . . In spite of all efforts the final diagnosis might still be clouded in doubt”. It is not surprising in view of the uncertainty of accurate diagnosis that constant attempts have been made to improve the results of conservative treatment for traumatized pulps and the first of these turned to antibiotics. James, Englander and Massler\(^{19}\) however did not attain much success using antibiotics alone, although inflammatory response was less. Shroff\(^{20}\) was of the opinion that calcium hydroxide preparations are the most successful and tubular dentine is formed where less irritation has occurred. Seelig\(^{21}\) working on monkeys, supports Shroff's opinion. He also noted that chloretetracycline resulted in tissue destruction and the formation of localized abscesses. He noted that dentine chips act as a nidus for calcification and this was found in the present study (Fig. 10). When the pulp was not infected, the application of penicillin in association with dentine chips was followed by calcification. Seelig was of the opinion that pulp-capping is a hit or miss procedure, and in an infected pulp a dentine bridge may be formed at one point and an abscess at another, and he emphasized that carious or contaminated dentine should not be allowed into the pulp.

Kakehashi, Stanley and Fitzgerald\(^{22}\) demonstrated the important role of infection plays in the repair reaction of the pulp and that, in the absence of infection (germ-free animals) and without any capping procedure, calcific repair with dentinal bridging may occur in 14 days. This may be compared with our observations (Table 1 and Fig. 1).

Kiryati\(^{23}\) and Koslov and Massler\(^{24}\) found some evidence of calcific repair when corticosteroids were used on traumatized rat molars, and Schroeder and Triadan\(^{25}\) found that in 200 patients, aged 15 to 24 years, in which pulps were exposed, carious dentine removed and a paste of triamcinolone, chloramphenicol and lignocaine in an ointment base applied, pain disappeared in 2 to 3 hours. The dressing was removed after seven days and replaced with zinc oxide and eugenol plus one third

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weight of triamcinolone. They found vacuolization in pulps of both treated and untreated teeth and they recognized the necessity for some antibacterial agent. Fry, Neely, Ruhman and Phatak\textsuperscript{(20)} found in a group of patients, aged 10 to 64 years, where a glucocorticosteroid compound without calcium hydroxide had been applied, that in 300 teeth at the end of 18 months 51 had periapical lesions. Nevertheless the control of pain was an important result.

Lawson and Mitchell\textsuperscript{(20)} treated 50 cases of painful pulps with a pulp-capping compound consisting of erythromycin, streptomycin plus a glucocorticosteroid, and reported no clinical failures after an average interval of 91 days; one half of the control teeth treated with starch failed. Baume\textsuperscript{(22)} in a further report showed that histological evidence did not corroborate the favourable clinical and roentgenographic evidence. He noted the absence of a solid barrier at the site of trauma, arrested dentine formation, residual or induced chronic inflammation even after long post-operative intervals. He did not find a single case showing solid barrier formation and “there was an increased amount of necrobiotic change”. Ehrmann\textsuperscript{(22)} found that five teeth, where exposure had been capped with a glucocorticosteroid, antibiotic calcium hydroxide compound, were vital and symptomless but in no case had the exposures been bridged by calcific repair and the pulps bled on probing.

Although repair dentine may be disclosed at roentgenographic examination this does not mean that a completely continuous calcific barrier exists.

The evidence presented by various writers supports the claims that the use of glucocorticosteroid compounds suppresses rapidly painful symptoms. Our results support this. The observations made by several writers that calcium hydroxide provides a more satisfactory calcific barrier is not wholly supported by the evidence presented in this paper. Figure 5 shows that on serial section at higher magnification the calcific barrier is not complete. However, it is important to recognize that this report presents the results obtained from the limited number of teeth available. It has indicated the need for more extensive investigation, especially when it is remembered that suppression of inflammation deprives the tissues of an active defence mechanism. The inability to maintain complete integrity of the protecting walls of the pulp chamber is a major defect and in certain situations and conditions could be dangerous to the health of the patient. Klotz, Gerstein and Bahn\textsuperscript{(2)} have demonstrated bacteriemia after topical use of a glucocorticosteroid placed over exposed and infected pulps in the molars of monkeys. Since the addition of antibiotic and calcium hydroxide does not ensure complete calcific barrier, this danger remains. In the presence of a resistant strain of bacterium suppression of inflammatory reaction in young teeth with good blood supply and patent apices provides a potentially dangerous situation for the development of a transient bacteriemia, particularly in the patient with a bacterial endocarditis.

The large venous sinuses seen in the pulp are a matter of interest and at this time it is not possible to do more than speculate on their presence. One feature is that they were more numerous in the pulps treated with glucocorticosteroid. It has been demonstrated that certain chemical medication, such as histamine, serotonin and bradykinin, induce small vessels to leak fluids into the tissues. Inflammation induces an increased blood supply and, if prolonged, stasis develops. The histamine released by the damaged cells has activated the mechanism which permitted increased permeability. The glucocorticosteroid appears to control this permeability and the vessels remain enlarged. The absence of blood cells from the sinuses is not adequately explained. The presence of sinuses, in some examples occupying large areas of pulp, suggest degenerative changes and in the absence of adequate calcific repair barriers are a potential hazard which must be recognized.

Conclusions

1. The use of glucocorticosteroid compounds in the treatment of the traumatized human pulp does eliminate pain and may reduce inflammation.

2. Calcific repair occurs with calcium hydroxide. Dentino genesis ceased although some amorphous calcific material was seen when glucocorticosteroid was used. Neither were shown to develop a complete calcific barrier.

3. Areas of chronic inflammation persisted in pulps treated by both procedures, although to a lesser number and extent in the case of the use of glucocorticosteroids. The absence of pain may be an indication of the degenerative processes associated with the large venous sinuses.

4. There is a possibility that the glucocorticosteroid compounds, despite the incorporation of antibiotics and calcium hydroxide, may in certain conditions leave a vulnerable focus especially for a patient with bacterial endocarditis.

5. The necessity of a surgically clean procedure, including the use of rubber dam and avoidance of contamination of the pulp, must be emphasized.

6. More extensive study and greater numbers of teeth will be required to elucidate this problem. The use of the material needs to be tempered with caution until more definitive studies can be completed.

Summary

1. The effect on the healing of traumatized pulps in 44 teeth recovered from white rats, when glucocorticosteroid compounds and calcium hydroxide were used over a period of 2 to 22 days, has been examined.

2. A similar study was made on 15 teeth from children aged 14 to 16 years for 23 to 314 days.

3. It was observed that evidence of calcific reaction was seen in more than 50 per cent of teeth of the children in both treatments.

4. The calcific repair was not a complete barrier and in the case of glucocorticosteroid did not occur.

5. Pain was eliminated in all cases where glucocorticosteroid was used.

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Further observations on pulp reactions to a tetracycline compound

R. Harris
with the technical assistance of Christa Lossin
Further observations on pulp reactions to a tetracycline compound*

R. Harris †
with the technical assistance of Christa Lossin

Introduction

A previous report(1) indicated the nature of the reaction of the human pulp observed in a small series of teeth in which treatment had been performed on the pulp subsequent to its exposure in either carious or non-carious teeth. In addition, observations on the reactions of the pulps of rat molars were reported. In both series, after treatment, the cavities were sealed with amalgam and the teeth extracted at various intervals. Further material has been obtained from both human and rat molars in which the extent of penetration of tetracycline through dentine and pulp was studied.

Tetracycline is used as a means of identification in calcification and its incorporation in pulp capping material may be of use in demonstrating the degree of penetration of such agents through dentine and into the pulp.

The observations noted(2) in rat molars that had been treated with pulp-capping agents suggested that more detailed observations were warranted. This paper reports observations on the degree of penetration of these agents as illustrated by the use of tetracycline in the molar teeth of humans and rats.

Material and methods

The pulp-capping medium ‡ contains a tetracycline in association with other agents and was applied to the pulp in the form of a cement and sealed in position with amalgam for varying periods of time. The extracted human teeth, or in the rat the teeth and jaw segment, were immediately placed in formalin saline fixative. In the human teeth bur cuts had been carefully made into the root and cervical dentine to increase the rate of penetration of fixative. The tissues were then passed through increasing concentrations of alcohol up to 100 per cent and then transferred to acetone, embedded in Bioplastic (Ward's) and sectioned on a Gillings-Hamco ground-sectioning machine at approximately 100 microns thickness, mounted on slides with Eukitt mounting medium and examined under tungsten light and fluorescent light using Blue BG38 and BG12 filters with blue absorption suppression filter. The dispersion of the medium through the pulp was detected at various intervals after its application to the dentine and pulp.

Observations

1. Rat molars

An example of the fluorescence of the pulp tissue is shown in Fig. 1, 2, where the tetra-

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† Director, Institute of Dental Research, Sydney.
‡ Lederminx, a product of Cynamid International, Lederle Division.
cycloheximide substance had been applied for 20
minutes to a pulp previously exposed with
a fissure bur. It will be noted that the
fluorescence extends to the apical region in
both the distal and central roots. The mesial
root in toto is not recorded, the apical portion
not having been included in the section prepara-
tion. In the central root it is apparent that
the substance has penetrated beyond the
immediate root canal region into the dentine
and the apical cementum and some of the
periapical bone.

In another animal the cavity was prepared
and the pulp-capping material was sealed with
amalgam and recovery of the tooth from the
upper jaw followed after an interval of 21 days.
Two days subsequent to sealing and two days
prior to recovery of the tissues tetracycline
(5 mg./Kg. body weight) was injected into the
peritoneum.
Fig. 5.—Fluorescence in distal root of lower first molar of rat [16]. Note line of fluorescence in dentine and alveolar bone and degree of persistence of fluorescence. Same conditions as in [5]. × 22.

Fig. 6.—Fluorescence in coronal dentine of upper second molar (pulp exposure with caries) in patient aged 14 years. Pulp exposed and Ledermix cement applied for 80 minutes prior to extraction. × 16.

Fig. 7.—Fluorescence of periapical soft tissues of palatal root. Same patient as in Fig. 6. × 16.

Figures 3 and 4 show two examples of the presence of fluorescence within the coronal pulp and into a portion of the distal root after 21 days. Figure 5 is from another animal in which the material had been sealed in a lower right molar for 21 days and tetracycline injections were also given to the animal. The fluorescence has again largely dispersed from the main portion of the root. In Fig. 3, 5, tetracycline injections show that growth of dentine has continued subsequent to the treatment to the pulp.

2. Human molars

A number of patients were selected from whom teeth were to be extracted at short notice. In each tooth the carious dentine was
removed, a pulp exposure made and the tetracycline material sealed into place. Two examples of the fluorescence reaction are shown in Fig. 6-12.

Case i: The upper second molar of a child aged 14 years

Tetracycline compound applied to the pulp and sealed. Tooth extracted 50 minutes later. Figure 6 shows the extension of the tetracycline through the dentinal tubules and in some portions it has travelled the full extent of the dentine and appears to have concentrated at the pulpal dentine junction. On further examination it will be seen (Fig. 7) that fluorescence is present in the apical cementum and in the periapical soft tissues.

Case ii: The upper left second molar of a child aged 16 years

Tetracycline compound applied to the pulp and sealed. Tooth extracted 80 minutes later. Figure 8 shows a section of the pulp of the palatal root with a blood vessel lying longitudinally and parallel to the dentine wall. When illuminated by fluorescent lighting the section
(Fig. 9) shows marked fluorescence of this vessel and the apical region of this root is shown similarly in Fig. 10, where fluorescence persists at the apical pulp and periapical region. Figures 11 and 12 show under similar conditions the extent of penetration of the tetracycline through pulpal vessels and the periapical space in the mesial root of the same tooth. It will be observed that the penetration through the soft tissues and to the periapical space has reached the same degree as in the palatal root.

Discussion

The use of tetracycline in labelling the progress of calcification in bone and dentine is well documented; most recently by Antalovska in which he demonstrated fluorescence in long bones, alveolar bone, enamel and dentine of the incisors but not in that of the molars in rats. This fluorescence persisted for a period of up to ten weeks. However, our observations show some effect in the dentine of molars following tetracycline injection.

Falck and Falck, Hillarp, Thieme and Torp have also used a fluorescence technique for demonstrating the presence of certain monamines in tissues, and more recently Waterson and Smale and Waterson have demonstrated fluorescent structures in blood vessels of the rabbit's ear and pulp. These observations depend upon the fluorescence of noradrenaline containing granules in adrenergic nerve terminals confined to the outer border of the smooth muscle layers of the arteries when treated with formaldehyde gas.

Götze reported the degree of penetration of tetracycline through the human tooth over a range of two hours to five days. The teeth had been diagnosed as undergoing pulptis and the carious lesions were treated by cavity preparation followed by the placing of Ledermix in the cavity. Of the 15 teeth he examined five had exposed pulps.

From the observations of the reaction in the rat molar it can be seen that there is a marked fluorescence early and extensively throughout the pulp. This indicates that there is a rapid spread of the tetracycline component through the dentine and pulpal tissue. As the interval between application and recovery extends removal of some of the material in the apical regions of the pulp occurs (Fig. 3, 4).

It is of importance to note that the extension of the fluorescence through the dentinal tubules occurs and that in the human tooth it reaches the pulpal surface of the dentine in a short space of time. Furthermore this degree of penetration is sufficient to extend through the pulp to the periodontal tissues at the apex of the root. Götze noted that following pulp capping in teeth with pulptis fluorescence appeared throughout the pulp and persisted for as much as 3-5 days. He was of the opinion that the dentine barrier slowed down absorption and where carious dentine was present that penetration did not take place.

In the rat molar it is shown that subsequent to "pulp capping" dentine growth continues (Fig. 3, 4, 5). It is important to note that the exposures in these teeth were treated immediately and therefore pulpitis would be minimal or non-existent.

In the molar teeth from humans tetracycline appears to have passed rapidly through the pulp and into the periapical tissues (Fig. 6-12).

The clinical implications of these observations are important, because if the constituents of the pulp-capping material remain in the pulp it may be reasonably expected that its effect may persist for some time. Provided calcific repair follows, the end result could be satisfactory.

The continuation of deposition of dentine as shown by that following the first tetracycline injection suggests that this result is possible. That it does not always occur may arise from the unfavourable conditions existing in the pulp at the time of treatment.

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The ultrastructure of the blood vessels of the human dental pulp following injury. I.

C. J. Griffin and R. Harris
The ultrastructure of the blood vessels of the human dental pulp following injury. I.

C. J. Griffin* and R. Harris†

Introduction

This paper is the first of a series of electron-micrographic studies of the reactions of normal human dental pulps exposed during cavity preparation and treated in various ways.

In the first instance we wished to examine the reactions to injury of the normal pulp after it had been covered with inert material and sealed from the oral cavity and subsequently reactions following the use of calcium hydroxide, and a corticosteroid as capping agents.

In this paper we report the observations made on the human dental pulp recovered from normal teeth two days after its exposure during cavity preparation.

Materials and methods

The two specimens used in this study were obtained and prepared, according to the method described by Harris and Griffin,1) from the teeth of patients aged 12 years. The pulps had been exposed during cavity preparation in premolars designated for extraction for orthodontic purposes.

The tooth was placed under rubber dam and the pulp was exposed with sterile instruments; when bleeding had ceased any blood clot was gently washed away with sterile normal saline, the cavity dried, the pulp covered with a small piece of soft gutta percha, and finally the cavity was sealed with amalgam. The patient was instructed to avoid the tooth during eating and to return in two days or earlier if pain was experienced. Both patients returned at the two days' interval and neither complained of pain.

Observations

Inflammatory changes, dilated blood vessels and diapedesis had occurred in the coronal portion of the pulp. Erythrocytes were present in the cytoplasm of the endothelial cells, lying between the basal lamina of the endothelial cells and the pericytes (Fig. 1) and in the extracellular substance surrounding the vessel (Fig. 3).

Numerous smooth-walled vesicles were present in the cytoplasm of the endothelial cell and some were found in the basal lamina (Fig. 1). In some instances the plasma membrane of the endothelial cell projected into the lumen of the blood vessel where, in some situations, it was associated with an erythrocyte. The basal lamina appeared to be split to enclose pericytes of diverse structure (Fig. 1). The cytoplasm of some of these pericytes contained a few ribosomes and mitochondria with irregular cristae and a pale matrix (Fig. 1); in other instances numerous ribosomes, grossly dilated rough surfaced cisternae containing fine filamentous material, and swollen mitochondria with irregular cristae in the cytoplasm were present. Large vacuoles were seen in the endothelial cells and the pericytes.

More detailed examination of the capillary endothelial cells showed in some instances discontinuity in the basal lamina and fragmentation of the cytoplasm, and erythrocytes were seen to protrude into the zone of interruption of the wall of the blood vessel (Fig. 2).

The extracellular material consisted of a few collagen fibrils and filamentous material, and the processes of phagocytic cells were near the vessel. In other regions leucocytes, erythrocytes, and monocytes with indented nuclei, pinocytotic vesicles, a few profiles of rough surfaced endoplasmic reticulum and myelin figures were seen (Fig. 3). Cells containing a nucleus and having a Golgi complex, dilated cisterna, lipid droplets and rough surfaced endoplasmic reticulum were identified as fibroblasts. Neural elements close to the blood vessels had undergone degenerative changes. The mitochondria of the Schwann cell had sparse irregular cristae and there appeared to have been severe shrinkage of the axoplasm of certain of the myelinated nerve fibres (Fig. 4). Associated cells, presumably Schwann cells because of their basal lamina, had pyknotic nuclei.

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Fig. 1.—Dilated capillary from coronal portion of exposed pulp.  
E, erythrocyte partially enclosed by endothelial cell membrane;  
Eₚ, erythrocyte in cytoplasm of endothelial cell;  
N, pyknotic nucleus of pericyte;  
l, lumen;  
c, cytoplasm of endothelial cell;  
v, vesicles in endothelial cell;  
pₑ, pericyte with clear cytoplasm;  
pₑᵗ, pericyte with numerous ribosomes;  
b, basal lamina of pericyte;  
arrows indicate fragmentation of endothelial cell wall.  
×11,400.

Fig. 2.—Disruption of endothelial cell wall of a dilated capillary.  
E, erythrocyte;  
p, phagocytic cell;  
c, cytoplasm of endothelial cell;  
evₓ, extravascular substance;  
l, lumen;  
arrows indicate interruption of endothelial cell wall.  
×22,400.
Fig. 3.—Phagocytic cells and extravascular substance. N, nucleus of monocyte; E, erythrocyte; L, leucocyte; F, fibroblast; ecs, extracellular substance. ×15,600.
Fig. 4.—Neural elements associated with inflammatory process. a, axon of myelinated nerve fibre with little evidence of shrinkage; b, axon showing gross shrinkage; N, pyknotic nucleus of Schwann cell; m, degenerate mitochondrion; am, amylated nerve fibre; e, terminal enclosed axons; f, terminal exposed axons. ×11,400.
Inset (1) enclosed nerve endings, (2) exposed nerve endings. ×34,200.
Amyelinated nerve fibres and two types of nerve endings could also be identified; in one, terminal axons were exposed to the extracellular substance, and in the other the nerve endings were enclosed by Schwann cell cytoplasm. The nerve endings usually contained small mitochondria and synaptic-like vesicles.

Discussion

There does not appear to be any reference to electron microscopy of the early inflammatory changes in the dental pulp of human teeth.

Novák and Merker, however, did study the canine pulps of dogs 24, 48 and 72 minutes after interrupting the blood circulation and found no significant changes in the structures of the intercellular space of fibrous tissue and of the connective tissue cells; there was an increase in the number of vesicles in the endothelial cells according to the duration of the interruption and the myelin sheath of nerve fibres was altered after 48 minutes.

Novák, Merker and Kvapilová studied alterations in pulps of dog's teeth that had been exposed to arsenic trioxide. The first changes could be observed after three hours in the coronal pulp; fragmentation, shrinkage, and an increased number of neurofilaments in the axons were noted. The plasma membranes of the endothelial cells had disintegrated and a protein-rich oedema had developed. At longer intervals deeper portions of the pulp were similarly affected.

In our study we noted evidence of inflammatory changes in the coronal pulp 48 hours after trauma. The results are similar to the light microscopy findings of Selzter and Bender, namely that pulp exposure produces severe inflammatory changes.

Capillary vessels become dilated and erythrocytes penetrate the cytoplasm of endothelial cells and gain access to the extravascular tissues (Fig. 1, 3). It would appear that initially dilated capillary vessels are associated with stasis. Because of this, the erythrocytes adhere to the luminal plasma membrane of the endothelial cell. Subsequently processes of the endothelial cell appear to envelop the erythrocyte and it passes into the cytoplasm by a process of macropinocytosis. Alternatively, erythrocytes may escape from the blood vessels by enlarged endothelial pores (Fig. 1). The nuclei of the endothelial cells become pyknotic and the cytoplasm has large vacuoles and degenerate mitochondria. Frequently the endothelial cell wall was seen to be fragmented and interrupted (Fig. 2) and erythrocytes were seen protruding into the endothelial pores.

A prominent defence reaction was seen by the presence of phagocytic cells, monocytes, and leucocytes in the extracellular tissues (Fig. 3). The extracellular substance was principally of a granular nature and only a few collagen fibrils were present compared with those seen in the normal pulp by Harris and Griffin. Erythrocytes, phagocytic cells, cellular debris, denatured collagen fibrils and granular elements were present in the extracellular substance. These elements constituted a protein-rich oedema.

Certain changes were seen in the neural elements, although we could not be certain that the changes were not caused by the fixation procedures. These changes consisted of axonal shrinkage, pyknotic nuclei of the Schwann cells and degenerate mitochondria in the cytoplasm. The fact that some of the myelinated nerve fibres showed little evidence of axonal shrinkage, whilst in others it was gross, suggests that the changes were not fixation artefacts. Similar changes were demonstrated by Novák, Merker and Kvapilová in the neural elements of the dog canine pulp three hours after the application of arsenic trioxide and similar changes had been demonstrated by Novák and Merker 48 minutes after obstruction of the pulp circulation.

An unique feature of the results in this study of early inflammatory changes in the dental pulp is the increase in the ribosomal content of the cytoplasm and the grossly dilated cisternae of the endothelial cells (Fig. 1). This suggests that one of the reactions of endothelial cells is an increase in the synthesis of protein which may contribute to the protein-rich oedema associated with trauma.

Summary

Electron microscopy of the exposed normal human dental pulp capped with gutta percha for two days revealed diapedesis of erythrocytes, grossly dilated cisternae and increased ribosomes of the cytoplasm of endothelial cells, and some axonal shrinkage of myelinated nerve fibres. Degenerate extracellular substance and phagocytic cells contributed to a protein-rich oedema. Some erythrocytes were seen in the endothelial cytoplasm.

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The ultrastructure of the blood vessels of the human dental pulp following injury. Part II

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Introduction
Observations have been recorded(1) on the changes demonstrated in electronmicrographs in the normal dental pulp subjected to trauma by exposure during cavity preparation and then covered with gutta percha and sealed with amalgam for a period of two days.

The principal changes noted were diapedesis of erythrocytes, some of which had entered the endothelial cytoplasm, grossly dilated cisternae, an increase in the number of ribosomes in the cytoplasm of the endothelial cells, and some axonal shrinkage of myelinated nerve fibres.

This paper reports observations made after a period of four days on pulps treated in a similar manner compared with the changes demonstrated when the exposed pulp had been "capped" with a glucocorticosteroid compound (Ledermix cement).

Materials and methods
Four specimens were obtained and prepared according to the method described by Harris and Griffin.(2) The premolars had been selected for extraction in the course of orthodontic treatment.

The procedures of cavity preparation, "pulp capping" and restoration were completed with sterile instruments and rubber dam as described previously(1) and two pulps were protected with a glucocorticosteroid (Ledermix cement). The patients were instructed to avoid the teeth during eating and to return at the end of four days or earlier if pain occurred. The patients reported freedom from pain.

Observations
Four day exposure of pulp
The coronal part of the pulp showed degenerative changes similar to those seen after two days.(1)

The capillary blood vessels appeared to be dilated and the ground substance consisted of granular material with few collagen fibrils (Figs. 1, 2).

Complex changes were observed in the endothelial cells of the capillaries. The cytoplasm contained many profiles of the rough surfaced endoplasmic reticulum, pinocytotic vesicles and degenerate mitochondria. Large vacuoles were also seen in the cytoplasm; the density of the cytoplasm in these cells, in part, appeared to be due to the presence of free ribosomes formed into rosettes. In some instances a few degenerate mitochondria and other organelles were seen in the cytoplasm. Such cells had a lashed appearance. The processes of pericytes were markedly dense and contained large vacuoles and the basal lamina appeared to be undergoing disintegration in certain areas.

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Fig. 1—Capillary of the transected pulp with an erythrocyte in the lumen. L, active endothelial cell; Lc, lymphocytes; R, red blood cell; V, vacuole; P, pericyte. Original magnification x30,000.
Fig. 2—Dilated capillary of the granular pulp. E, endothelial cells; N, nucleus of pericyte (e, electron dense, double line); G, granular extracellular substance; c, collagen fibrils; h, histol. 

with that of the nucleus of an endothelial cell. 

Oxyradial lamina.
Fig. 4.—Neural tissue from traumatized pulp. N, pyknotic nucleus of Schwann cell; pc, dilated perinuclear cisternae; a, axon; m, electron dense mitochondria. Fulde ×15,000.

Fig. 5.—Detail of Fig. 4; mv, multivesicular body; S, Schwann cell cytoplasm; a, axon. ×80,000.
Similar characteristics were seen in the endothelial cells and smooth muscle cells of small arterioles. The smooth muscle cells contained huge vacuoles, degenerate mitochondria, and myelin figures in the cytoplasm. The extracellular substance appeared to be normal (Fig. 3).

Neural tissues appeared to be affected to some degree. The nuclei of Schwann cells were pyknotic and showed grossly dilated perinuclear cisternae containing fine granular material. Multi-vesicular bodies were present in the cytoplasm of the Schwann cell and axonal mitochondria were electron dense (Figs. 4, 5).

Ledermix protected pulp
The blood vessels in the pulps treated with Ledermix had undergone mild reactive changes.

Capillaries
The endothelial cells contained mitochondria with a moderately dense matrix, lipid droplets, pinocytic vesicles, electron dense bodies and a few profiles of the rough surfaced endoplasmic reticulum. Some ribosomes were seen in the cytoplasm. The pericytes had grossly dilated cisternae, mitochondria with regular cristae, electron dense bodies, pinocytic vesicles and autophagic.
Fig. 7.—Occluded terminal arteriole from medicated pulp. E, endothelial cells; sm, smooth muscle cell; b, electron dense bodies; m, mitochondria with regular crista; r, ribosomes; v, pinocytotic vesicles; ecs, extracellular substance. Enlarged ×10,000.
vacuoles (Fig. 6). The extracellular substance appeared normal.

**Arterioles**

The terminal arterioles were occluded. The endothelial cells were cuboidal in shape and their mitochondria had regular cristae. However, the cytoplasm contained numerous pinocytotic vesicles and several electron dense bodies. The ribosomal component of the cytoplasm was markedly increased.

The smooth muscle cells appeared to be essentially normal, the mitochondria had regular cristae, and the myofilamentous components appeared normal (Fig. 7).

**Discussion**

Apparently the traumatized area of the untreated pulp very quickly undergoes degenerative changes consisting of necrosis of cells and dissolution of the ground substance (Figs. 1, 2, 3). Areas peripheral to the trauma also exhibit signs of degeneration. The most striking feature is the change in the endothelial cells of the blood vessels which show in some instances an enormous increase in their ribosome components. It would seem that these cells initially react with increased protein synthesis and thus contribute in part to an extracellular protein-rich oedema. Associated with this is an increase in the number of pinocytotic vesicles. Some endothelial cells, however, undergo lysis.

In general, changes in the exposed pulp after four days are not markedly different from those seen after two days.\(^{1}\)

Where the corticosteroid has been applied, the inflammatory reactions are more subtle. There appears to be almost complete occlusion of the terminal arterioles (Fig. 7). Because of this the capillary blood vessels are not obviously dilated (Fig. 6). Diepsedesis, emigration of leucocytes, and changes in the extracellular substance were not observed. Nevertheless certain changes occur in the cellular components of the blood vessels.

Lipid droplets, electron dense bodies, and pinocytotic vesicles are present in the cytoplasm of the endothelial cells and, in general, their mitochondria have regular cristae and the matrices were moderately electron dense.

The smooth muscle cells appear to be normal. Compared with the normal blood vessels of the pulp previously described,\(^{12}\) there is an increase in certain of the cytoplasmic components. This increase is mainly due to the number of free ribosomes in the cytoplasm. The increase, however, is not nearly as marked as that observed in the traumatized but unprotected pulp. There is also an increase in the number of pinocytotic vesicles. This pattern also applies to the structure of the pericytes associated with the capillaries. No degeneration of basal lamina was observed.

It would appear, therefore, that the main effect of the corticosteroid (Ledermix cement) is to reduce hyperaemia by the occlusion of terminal arterioles and thus prevent or minimize initial inflammatory changes.

**Summary**

Observations made on the effect of exposure on human dental pulps and modification of these effects by medication four days after injury show in the former extensive reactive changes with an increase in cytoplasmic components, large vacuoles and breakdown of basal lamina in cells of the blood vessel walls, together with migration of leucocytes and erythrocytes; in the latter the changes are much less marked and there is almost complete occlusion of the terminal arterioles, an increase in pinocytotic vesicles and ribosomes and an absence of migration of erythrocytes and leucocytes was noted.

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The ultrastructure of the human dental pulp following injury. Part III. Extravascular tissues

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Introduction
Previous papers in this series have reported observations on the ultrastructure of the blood vessels of the human dental pulp\(^{(1)}\) and of the blood vessels at intervals of two\(^{(2)}\) and four\(^{(3)}\) days subsequent to injury and medication. The final two papers report observations after an interval of fourteen and twenty-one days. The observations reported in this paper are confined to the extravascular tissues in the central region of the traumatized pulp.

Method
The procedure followed the method outlined\(^{(1)}\) in the first paper and using bicuspids teeth designated for extraction, as part of orthodontic therapy, Ledermix cement was used as the medicament applied to the exposed pulp.

The material was obtained from two teeth from a patient aged 12 years.

Observations
Central traumatized area
The tissue immediately underlying the traumatized area showed sterile necrosis. Certain areas of cells have undergone complete disintegration so that nuclear and cytoplasmic fragments were present surrounded by areas devoid of ground substance, a few collagen fibrils and clumps of apparently denatured collagen fibrils (Fig. 1, 2).

The isolated nuclei had pyknotic zones and the outer nuclear membrane was disrupted and only fragments of cytoplasm remained (Fig. 2). The denatured collagen fibrils appeared as electron dense clumps with no signs of periodicity except for a few fibrils. No remnants of the ground substance microfibrillar reticulum (beaded microfibrils) were observed.

In other areas (Fig. 3) fibroblasts undergoing lysis were present. The nuclei of these cells were markedly pyknotic and huge vacuoles with occasional lipid droplets were present in the cytoplasm. The plasma membrane was frequently disrupted. The extracellular substance contained a
few sparse collagen fibrils and some clumping of denatured collagen and the microfibrillar reticulum was absent from the ground substance.

**Reactive complex**

Surrounding the area of necrosis were fibroblasts and numerous macrophages (Fig. 4, 5, 6). The fibroblasts were seen to have swollen mitochondria and numerous profiles of the rough surfaced endoplasmic reticulum (Fig. 4, 6 (a)). The nuclei of the cells were slightly pyknotic and pinocytotic vesicles were seen in the cytoplasm. Collagen fibrils were associated with the plasma membrane of these cells, although there was scant evidence of the microfibrillar reticulum in the ground substance.

As noted above, by far the most common cells surrounding the central area of necrosis were macrophages (Fig. 4, 5, 6 (b)). Usually, the mitochondria of these cells had a normal appearance with regular crista mitochondriales. The most significant aspect of the cytoplasm was the presence of numerous pinocytotic vesicles. These vesicles appeared to be undergoing coalescence with membrane-bounded bodies to form primary and secondary lysosomes.

The plasma membrane of the macrophages was occasionally sectioned obliquely, in which case
collagen fibrils were seen to be intimately associated with it. In other areas where oblique sectioning was less obvious, collagen fibrils appeared to be incorporated in the cytoplasm (Fig. 6 b)). Sometimes what appeared to be fragments of collagen fibrils were present in the pinocytotic vesicles.

Discussion
We have confined our observations in this paper in order adequately to report the changes noted in the area immediately adjacent to the traumatized zone of the pulp. The significant changes observed were necrosis and pyknosis of fibroblasts, together with denaturation of collagen fibrils and destruction of their associated microfibrillary reticulum.

The changes observed support the contention that attached reticular fibrils are necessary for the maintenance of collagen fibrils and we have previously postulated that elaboration of this reticulum was essential for fibrillogenesis to occur.101 We have also demonstrated this reticulum is labile to beta-

Fig. 3.—Tissue from traumatized area of pulp. N, pyknotic nucleus of fibroblast; v, vacuole containing lipid droplets; arrow, clumping of collagenous material. Note the expanse of collagenous tissue and absence of ground substance microfilamentous reticulum. Palade ×11,880.

Associated with the enzymatic destruction reticulum clumping of denatured proteinsaceous tissue was observed. This reticulum has also been identified in the ground substance of the periodontal membrane.

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Fig. 6.—(a) Fibroblast and macrophage associated with denaturation of ground substance. N, nucleus of fibroblast; or, rough surfaced endoplasmic reticulum; pv, phagocytic vesicles; ec, extracellular substance devoid of collagen fibrils and microfilamentous reticulum. Palade ×12,300. (b) Details of macrophage. N, nucleus; pv, phagocytic vesicles; ec, extracellular substance; arrows, lyosomal fragmentation and absorption of collagen fibrils. Palade ×19,200.
It would appear that this microfibrillar reticulum is a component of normal dental pulp and periodontal tissues and for that matter all collagenous ground substance. This would seem to exclude the possibility that the microfibrillar reticulum is a contamination of the tissues due to exudation of blood plasma.

The assemblage of macrophages around the necrotic area is significant. These cells are actively engaged in phagocytosis of the necrotic tissue and indicate that local cortisone therapy does not interfere with a delayed defensive response.

The fibroblasts were undergoing lysis and disruption of plasma membranes was observed.

The possible reasons for pulp necrosis will be elaborated in the succeeding paper.

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The ultrastructure of the blood vessels of the human dental pulp following injury. IV

R. Harris and C. J. Griffin
The ultrastructure of the blood vessels of the human dental pulp following injury. IV

R. Harris* and C. J. Griffin†

Introduction

Previous papers in this series have reported observations on the ultrastructure of the blood vessels of the human dental pulp (1) and of the blood vessels at an interval of two, four, 14 and 21 days (2) (3) (4) subsequent to injury and medication.

The report on the 14- and 21-day conditions was confined to the traumatized and immediately adjacent areas. This present paper reports the state of the pulpal tissue distal and apical to the traumatized area.

Observations

1. Distal zone

Capillaries: The capillary blood vessels appeared to be essentially normal except for the presence of numerous electron-dense bodies lying in the cytoplasm of the endothelial cells (Fig. 1, 2, 3). Certain of the electron-dense bodies did not appear to be membrane bounded (Fig. 3), but occasionally they were seen to be vacuolated (Fig. 1, 2).

The cytoplasmic components otherwise appeared to be normal and mitochondria with regular and irregular cristae were present (Fig. 1). The cytoplasmic processes of the pericytes also appeared to be normal.

The basal laminae of these vessels were intact. The extracellular material consisted of collagen fibrils and fine beaded filamentous material (Fig. 1, 2, 3).

Arterioles: Most of the arterioles were either completely or partially occluded (Fig. 4). The cytoplasm of the endothelial cells contained a few electron-dense bodies but otherwise had a normal appearance. Smooth muscle cells also contained electron-dense bodies and multivesicular bodies. The basal laminae of both the endothelial cells and smooth muscle cells appear to be normal. The extracellular substance contained collagen fibrils together with cell processes of macrophages and fibroblasts. Unmyelinated nerve fibres were seen in close proximity to the arteriole (Fig. 4). These nerve fibres contained electron-dense mitochondria and synaptic-like vesicles, some of which had electron-dense material were present in the exposed axons.

Odontoblasts: The cell bodies of avulsed odontoblasts distal to the traumatized area were seen to be joined together by junction complexes. The cytoplasm of the odontoblasts contained lipid droplets sometimes enclosed in vacuoles, a few dilated profiles of the rough surfaced endoplasmic reticulum and autophagic vacuoles; fine filaments were also present but free ribosomes were not identified. Körf's fibres and cell processes were present in the intercellular spaces (Fig. 5).

The mitochondria showed various stages of disintegration; they contained regular and irregular cristae and others were swollen with only a few irregular cristae. Some cells had undergone complete

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lysis and large vacuoles, mitochondria and other organelles were seen in the extracellular substance.

(2) Apical zone

The terminal arterioles in the radicular region were usually occluded. The cytoplasm of the endothelial cells appeared to be essentially normal and the smooth muscle cells also appeared intact. The extracellular substance consisted of dense collagenous tissue (Fig. 6). Many vesicles are present in the endothelial cell cytoplasm. Certain of the metarterioles were also occluded and some electron-dense bodies were present in the cytoplasm of their endothelial cells.

Capillaries were either patent or collapsed. The endothelial cells contained electron-dense bodies and large vacuoles were also present; some filamentous material was present in these vacuoles and there were dilated profiles of the rough surfaced endoplasmic reticulum in which some filamentous material was also seen. The extracellular substance consisted of dense masses of collagen fibres associated with a microfibrillar reticulum (Fig. 7).

Other blood vessels appeared to be undergoing degeneration. The mitochondria of the epithelial cells were grossly enlarged and had irregular cristae (Fig. 8 (a), (b)). The lumen of the vessels in some cases contain a fibrinous material (early thrombosis). There were few organelles but numbers of electron-dense bodies in the cytoplasm of the endothelial cells (Fig. 9 (a), (b)).

The extracellular material surrounding the vessels consisted of collagen fibrils, cytoplasmic processes of cells and numerous free electron-dense bodies which resembled the electron-dense bodies seen in the cytoplasm of some of the endothelial cells. In some situations these bodies were seen in the basal lamina of the capillary and in close proximity to the plasma membrane of the endothelial cells (Fig. 9 (b)).

(3) Myelinated nerve fibres

Most of the myelinated nerve fibres appeared to be normal but the cytoplasm of the Schwann cells contained numerous mitochondria with electron-dense matrices. Axonal shrinkage was observed but this possibly could be the result of a fixation artefact. Occasionally large vacuoles were present in the Schwann cell cytoplasm (Fig. 10).
Fig. 2.—Endothelial cell of capillary from Fig. 1. N, nucleus; l, lumen; b, electron-dense bodies; v, vacuole, with large electron-dense bodies; c, collagen fibrils; f, beaded filamentous material. Magnification ×18,800.

Fig. 3.—Capillary blood vessel (distal zone). N, nucleus of endothelial cell; f, beaded filamentous material; c, collagen fibrils. Note numerous electron-dense bodies in the endothelial cells. Magnification ×18,725. Inset, detail of electron-dense bodies. Magnification ×82,575.
Discussion

The results following injury to the dental pulp capped with an inert material showed characteristic inflammatory changes. However, when Ledermix cement was placed over the injured area and the tissues examined at four, 14, and 21 days after injury more subtle changes were observed.

The initial response seems to be occlusion of the coronal blood vessels and the absence of characteristic inflammatory changes, viz. hyperemia, diapedesis, and migration of leucocytes. Nevertheless, the endothelial cells even at this early stage contained numerous electron-dense bodies. Fourteen and 21 days after injury sterile necrosis was observed in the central areas surrounded by a reactive zone.

Some areas of both the reactive and sterile necrotic zones were devoid of collagen fibrils and their associated microfibrillar reticulum. There was some evidence that this complex was undergoing phagocytosis by macrophages.

In the zone distal to the site of injury and the apical zone, responses, comparable with those in the central zone of pulps treated with Ledermix cement for four days, were observed. These changes suggest that eventually necrosis may occur in these areas.

Odontoblasts were clearly undergoing degenerative changes. Some cells had completely disintegrated whilst in others an internal reorganization (autophagia vacuoles) was present. The cells were distinctly different from those seen in normal pulps. Previously Harris and Bull and Barker and Ehrmann had noted reduction in size of the odontoblasts and their disappearance in pulps treated with Ledermix. More recently Barker, Payne, and Warby reported calcific repair in one of two teeth with Ledermix paste and cement.

Quite clearly the observations reported in this paper have been made on a limited number of teeth.

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Fig. 5.—(a) Cell bodies of avulsed odontoblasts. N, nucleus; arrows, functional complex; m, mitochondria in various stages of disintegration; K, Korf's fibres; l, lipid droplets. Palade ×13,500. (b) Portion of odontoblast showing internal reorganization. N, nucleus; arrows, functional complex; l, lipid droplets; a, autophagic vacuoles containing cytoplasmic organelles. Palade ×13,500. (c) Disintegrated odontoblast. Note presence of large vacuoles, mitochondria and cytoplasmic organelles in the extracellular substance. Arrow indicates remnants of plasma membrane. Palade ×13,500.
Fig. 6.—Ooccluded arteriole (distal zone). N, nucleus of endothelial cell; l, lumen. Palade ×10,200.

Fig. 7.—Collapsed capillary (apical zone). N, nucleus of pericyte; b, electron-dense bodies. N.B. the many vesicles in close proximity to the wall of the endothelial cells. Palade ×10,600.
Fig. 8.—(a) Patent capillary (apical zone). Note large numbers of electron-dense bodies in endothelial cells. Palade × 11,000. (b) Occluded capillary (apical zone). N, nucleus of endothelial cell; b, electron-dense bodies; l, lumen. Palade × 11,000.
Fig. 9.—(a) Examples of b, electron-dense bodies; m, enlarged mitochondria in endothelial cells. Palade $\times 15,000$. (b) Degenerate vessel (apical zone). Note grossly enlarged mitochondrion. l, lumen with fibrinous material; b, electron-dense bodies. Palade $\times 15,000$.

Note in both Figures electron-dense bodies in the extracellular substance.
and in the special circumstances where those teeth were sound and pulps were healthy before being subjected to the trauma and medication. However, this was essential in order that we might be better able to determine what reactions could be observed when the glucocorticosteroid plus tetracycline compound was applied to the pulp. The suppression of inflammatory reaction and the occlusion of small vessels would seem to have importance for the clinician.

There are conflicting reports(10) (11) (12) (13) on the histological reactions to the compound, but in general these can be grouped into those which suggest calcific repair does occur and those which report no repair. For example, Strömberg(13) stated the most striking features were a marked retardation of the healing of cortisone treated pulpectomies and partial pulpectomies.

Barker and Ehrmann(13) in a careful study reported observations on 12 teeth covering periods ranging from 29 to 727 days. Changes in the lumen of the canal and one case of internal resorption were noted but a complete dentine bridge was not formed in any of the teeth. They concluded that Ledermix “cement” applied to normal pulp tissue does not impair vitality and evokes a moderate calcific response after a prolonged period of contact.

Our observations support the findings of Ulmansky and Langer(10) and of Fiore-Damico and Baume(14) that in certain instances irreversible metaplastic changes occur in the pulp tissue at the site of contact with the cement which extend to variable depths.

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