CELLULAR ORGANIZATION AND PROLIFERATION IN THE DENTAL PULP

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CONTENTS

SUMMARY

Histological studies of the fully-developed human dental pulp have revealed the presence of subodontoblastic cell-free and cell-dense zones in the coronal pulp. These zones are not found in the root canals nor are they present in any region of developing pulps. Data is lacking on the comparative histology of pulps of other animal species.

The present investigation involved a study, by ordinary histological and radioautographic methods, of the organization and proliferative potential of cells in pulps of neonatal, young and old adult rats. An important aim was to examine the pulp tissues for the presence of structural layers in the subodontoblastic region comparable to the cell-free and cell-dense zones of the human pulp. As a preliminary to the main investigation, it was shown that the appearance of the cell-free and cell-dense zones in the human pulp does not represent an artefact resulting from histological processing of calcified and soft tissue in apposition.

The pulps of adult rats were found not to exhibit distinct subodontoblastic cell-free areas in any region. The suggestion has been made that the absence of a prominent subodontoblastic

cell-free zone in the rat pulp may be indicative of a less specialized sensory apparatus than in the human pulp and may also be of evolutionary significance.

Molar pulps showed a prominent subodontoblastic cell-dense zone in the roof and lateral aspects of the pulp chamber; such a zone was not present in the base of the coronal pulp nor in the root canals. The pulps of developing, unerupted molar teeth did not exhibit significant differences in cell density between the subodontoblastic and other regions.

The subodontoblastic cell-dense zone in the molar pulps developed during eruption of these teeth into the mouth and occurred by peripheral migration of cells from the internal regions of the pulp. These findings, together with data from radioisotope transfer studies, have led to the suggestion that exchanges between pulp and salivary fluids across enamel and dentine may mediate the cellular organization within the subodontoblastic zone of the rat molar pulp.

Results of the cellular proliferation studies using colchicine and tritiated-thymidine-radioautography showed very little mitotic activity in the incisor pulp in sites remote from the continually-growing basal region. Cells in the subodonto-

blastic cell-dense zone in the middle and incisal region were not observed to divide. Similarly, the cell-dense zone in the molar pulp was found to be a mitotically-inactive layer under normal conditions.

The possible functions of cells in the subodontoblastic zone have been discussed and the conclusion reached that present knowledge is inadequate for a full understanding of the biological significance of this cell-layer. A number of suggestions for future work have been outlined and some preliminary results presented on the rhesus monkey pulp which support the observations made on human and rat tissues.

SIGNED STATEMENT

This thesis is submitted as final fulfilment in the requirements for the Degree of Master of Dental Surgery in The University of Adelaide. Entry to candidature for the Degree was gained by passing a Qualifying Examination of a standard equivalent to the Honours Degree of Bachelor of Dental Surgery. The field of study for this examination was in Histology, Physiology and Pathology.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. Furthermore, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

Signed:

Theo Gotjamanos.

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PREFACE

A high incidence of dental disease exists among individuals of most civilized communities throughout the world. Irreversible pathological conditions of tooth and periodontal tissues dictate that a large proportion of clinical dental practice be devoted to restorative therapy. The institution of totally effective preventive measures is still mainly in the experimental stage because of inadequate knowledge of the factors involved in the maintenance of normal dental tissues and the initiation of disease. Nevertheless, dental research proceeds at an accelerating pace with the aim outlined by SOGNNAES (1959): "Prevention is the only rational solution, research the only hopeful approach", and toward the goal expressed by KAUFFMANN (1955): "The supreme ideal of the dental profession should be to eliminate the necessity for its own existence".

This study was undertaken with the aim of increasing the existing knowledge on the behaviour of cells in the normal dental pulp. It was hoped that such information might aid attempts to understand the role of this organ in maintaining the total health of the tooth and also help to formulate and stimulate further investigations into this important area of oral biology.

CHAPTER I

INTRODUCTION

Changing attitudes toward the biological importance of the dental pulp.

It was not until the beginning of the present century that the dental profession began to shown an interest in the role played by the dental pulp in maintaining the biological integrity of the tooth. The majority of clinicians of the nineteenth century had shown very little regard for conserving the vitality of the pulp and furthermore, did not consider it necessary to remove necrotic pulp tissue and seal the root canals (BLACK, 1934). According to BLACK, it was also a routine procedure to remove the healthy pulp tissue of every tooth which was to receive a crown restoration. This practice was based on the belief that the pulp of a fully formed tooth served no further useful function (TALBOT, LATHAM and ANDERSON, 1901) and the aim was to prevent the development of an inflammatory condition in the pulp following the operative procedure. In short, pulp conservation had no place in dental practice.

Many years passed before serious consideration was given to the possibility that the dental pulp might contribute to systemic infection. Following HUNTER'S historical lecture at McGill University in 1910 (HUNTER, 1911), in which he claimed that most

systemic illnesses were caused by oral sepsis, the dental profession immediately condemned countless numbers of pulpless teeth as focal points of infection. In time it became apparent that such radical treatment, apart from destroying the physiological function of the masticatory system, brought little, if any, relief for the systemic conditions. The dental profession next proposed the adoption of operative procedures which would promote the health and vitality of the pulp. Most techniques in operative dentistry were modified in order to minimize injury to the pulp (BLACK, 1934) and thus began the era of pulp protection and preservation.

During the past few decades many clinical and laboratory studies have been carried out on the response of the pulp to operative techniques. These investigations have led to the adoption of operative procedures that are biologically acceptable to the pulp (STANLEY, 1961). Clinicians of today regard the pulp as an essential and integral part of the tooth and all operative procedures are carried out in a manner which will result in a minimum amount of pulpal irritation. Conservation of pulp vitality is one of the most important aims of present-day dental practice.

odontoblast which participates in the formation of the organic matrix of dentine. HAN and AVERY (1962, 1963a) described the cytoplasmic organelles of the endothelial cells lining the pulpal blood vessels. AVERY and HAN (1961 a and b) reported observations on the ultrastructure of collagen in the pulp and showed that it possessed the characteristic 640 Å banding. In a further study, HAN and AVERY (1963b) studied the fine structure of pulp fibroblasts and their close association with collagen fibrils.

A few brief reports on histochemical studies of the dental pulp have appeared. ZERLOTTI (1964) demonstrated that the pulpal connective tissue is similar in its organization and histochemical reactivity to other connective tissues in that it contains in its extracellular matrix, glycoproteins, sialic acid, acid mucopolysaccharides and proteins bearing reactive epsilon-amino groups of lysine-hydroxylysine. This extracellular matrix was found to be organized as a heterogeneous colloid containing soluble and insoluble fractions, the latter increasing with age.

The activity of several dehydrogenases in the pulp during dentinogenesis was studied by DE SHAZER (1962) and FULLMER (1963) who found that the degree of activity of these enzymes

could be correlated with the degree of differentiation and function of pulp cells. Activity was highest during the early stages of development when dentinogenesis is most rapid. SCHWABE and KALNITSKY (1963, 1964) presented preliminary reports on the presence of acid proteases in bovine dental pulp and the possible role of a tissue dipeptidase in the terminal stage of collagen degradation. BHUSSRY and SPRINGER (1966) demonstrated an inverse relationship between the quantity of polysaccharide present and the degree of collagen fibre formation in the developing pulp tissue. The esterase component of human pulps was studied by RAPP, STRACHAN and AVERY (1965) who showed that four separate, individual esterases were present. In a further extension of this work, RAPP and STRACHAN (1966) demonstrated that all four esterases isolated were constituents of pulp tissue proper and were not derived from blood or plasma. A similar situation was found to exist in the pulps of primary teeth.

The concentration and distribution of acetylcholinesterase in pulps of primary and permanent teeth were compared by RAPP and AVERY (1964) in an attempt to obtain information which might explain the lesser sensitivity of primary teeth. Pulps of permanent teeth exhibited greatest amounts of acetylcholinesterase along

nerve trunks and blood vessels and in the cell-free and cell-rich zones. A similar pattern of distribution was observed in primary tooth pulps although the quantity of acetylcholinesterase present appeared to be considerably less than in permanent teeth.

Tissue culture studies of dental pulp cells have been undertaken by some investigators with a varying degree of success. The original work first carried out by GLASSSTONE and others on the in vivo cultivation of whole tooth germs has been supplemented by studies on isolated groups of pulp cells. ZUSSMAN and IOACHIM (1964) have reported the establishment of long-term tissue cultures containing odontoblast and mesenchymal cells obtained from the rat incisor pulp. POURTOIS (1965), using foetal rat material in tissue culture, investigated the inductive action of the enamel organ on the differentiation of odontoblasts from the mesenchymal cells of the dental papilla. He found that although the mesenchymal cells were capable of achieving a certain degree of differentiation in the absence of the enamel organ, the presence of the latter was necessary for the formation of a functional odontoblastic layer. POMERAT and CONTINO (1965) presented a preliminary report on the in vivo cultivation of mesenchymal cells obtained from human pulp tissue.

The vascular supply of the dental pulp has been studied with the aid of several injection techniques, and results have shown the pulp to be an extremely well vascularized tissue. The existence of a full anastomosis between the vessels of each root in multi-rooted human teeth was demonstrated by RUSSELL and KRAMER (1956) and KRAMER (1960) and in rat teeth by KINDLOVA and MATENA (1962). Numerous lateral canals connecting the root canal to the periodontal membrane were also observed. Furthermore, the work of KRAMER (1960) confirmed the observation of PROVENZA (1958) that arterio-venous anastomoses are present in the pulp. India ink-injected specimens of cat by CASTELLI (1962) showed a rich capillary network around the odontoblast cells, a fact also reported earlier by ADAMS (1959) in studies on rodent incisor pulps and by JAMES (1955) who studied ink-injected specimens of cat, hamster and rat. Similarly KINDLOVA and MATENA (1962) using latex instead of india ink observed a dense capillary network serving cells in the odontoblastic layer of the rat incisor and molar pulps. It is apparent from these studies that the odontoblastic cell layer is one of the principal vascular regions of the pulp.

The blood circulation in the dental pulp has been studied

only in an exploratory manner and little quantitative data is available. TAYLOR (1950) studied the characteristics of the blood flow in the rat incisor pulp directly via a 'window' (approximately 30 microns in thickness) prepared in the dentine. Utilizing the technique of TAYLOR, KOZAM and BURNETT (1959) determined the effect of thermal stimuli and several medicaments on the pulp circulation. Systemic changes were found to have marked effects on the pulpal blood circulation. POHTO and SCHEININ (1958 a and b) modified TAYLOR'S technique slightly and carried out observations on the circulation in the exposed and unexposed incisor pulp. In a continuation of this work, POHTO and SCHEININ (1962) studied the effect of local analgesic solutions on the pulpal circulation. These solutions, applied either as a continuous drip to a thin dentinal layer covering the pulp or administered as a mandibular injection, were found to cause a marked retardation of pulpal blood flow.

LIEBMAN and COSENZA (1962) used an electrical impedance dance technique to measure the rate of blood flow in the dental pulp of the dog and reported a volume of 0.06 ml per minute in the crown of the canine tooth. Further work with this method by NEIDLE and LIEBMAN (1964) involved a study of the effects of

vasoactive drugs and nerve stimulation on the pulpal blood flow in the cat. Results provided evidence that volume flow through the pulp is subject to wide variations as is blood flow in other parts of the vascular system. Intravenous administration of vasoactive drugs invariably produced changes in the volume flow through the pulp, and very often these changes paralleled those taking place in the systemic vessels. MEYER, WEINER and GRIM (1964) using an isotope fractionation technique measured the effects of anaesthetic and constrictor agents on pulpal blood flow in the canine tooth of the dog. They found that infiltration around the apex of the tooth with local anaesthetic solution had no significant effect on the pulpal circulation while 5 to 20 µg epinephrine deposited in a similar manner resulted in a decrease in the pulpal blood flow by about one-third of the value obtained for untreated teeth.

Measurement of the pressure within the pulp chamber of dog teeth was attempted by WYNN et al. (1963) who undertook a study to determine whether changes in the arterial blood pressure are reflected in the pulp chamber. Using the technique described by HALDI et al. (1961) of gaining access to the pulp chamber and by means of a pressure transducer, these investigators obtained

results which showed that a pressure exists within the pulp chamber and this pressure was altered following intravenous injection of adrenaline and acetylcholine, by cutting the vagi and by bleeding the animal. DELLOW and GOTJAMANOS (unpublished data) using similar techniques obtained an increase in pulp pressure of similar magnitude to that which occurred in the systemic circulation following intravenous administration of noradrenaline. A similar observation was reported by BEVERIDGE et al. (1964).

Analysis of dental pulp fluid was carried out by HALDI, WYNN and CULPEPPER (1961) who found that the average protein concentration in the pulp fluid was only about one-fifth of that in blood plasma. No significant differences in the inorganic element content and levels of glucose were noted. Electrophoretic studies by HALDI and WYNN (1963) showed that the pulp fluid contained the same relative amounts of protein fractions as were present in blood plasma. Further work by HALDI, LAW and JOHN (1965) established that the concentrations of chlorides, magnesium, urea and alkaline phosphatase in pulp fluid and blood plasma were the same but that levels of calcium and inorganic phosphorus were at a significantly lower level in pulp fluid than plasma.

Finally, in this brief review of pulp biology it is important to mention the radioisotope studies which have demonstrated the existence of exchange mechanisms operating between the pulp, the mineralized tissues surrounding it and the salivary environment, for the implications of some of the results obtained may have possible significance in the present study. In contrast to most of the other studies on pulp biology, this work has not been of recent origin. Some of these investigations were undertaken as soon as radioisotopes became available for biological use and were a natural extension of the experiments carried out previously by FISH, BODECKER, LEFKOWITZ and others. These early investigators had shown enamel and dentine to allow the outward and inward passage of dyes following application of these substances into the pulp or on to the tooth surface.

CHIEVITZ and HEVESY (1935, 1937) and HEVESY, HOLST and KROGH (1937) using radioactive phosphorus (P^{32}) showed that an exchange occurs between the phosphorus present in enamel and dentine with that in the blood. This observation with P^{32} was confirmed subsequently by several investigators, (MANLY and BALE, 1939; HEVESY and ARMSTRONG, 1940; VOLKER and SOGNNAES, 1941; WASSERMAN et al., 1941;

BARNUM and ARMSTRONG, 1942; GILDA, McCAULEY and JOHANSSON, 1943; McCAULEY and GILDA, 1943; BERGGREN, 1947; SOGNNAES and SHAW, 1952). Similar exchange mechanisms between the pulp and the calcified dental tissues were also demonstrated with the aid of other radioisotopes, including Ca^{45} (CAMPBELL, GREENBERG and MURAYAMA, 1940; PECHER, 1941; ARMSTRONG and BARNUM, 1948; FALKENHEIM, NEUMAN and HODGE, 1951; D'IORIO and LUSSIER, 1951; UNDERWOOD and HODGE, 1952; JARABAK, KAMINS and VEHE, 1953) and Na^{24} (KOSS and GINN, 1941; BERGGREN, 1947; SOGNNAES, 1957).

Exchanges between the pulp and saliva in rhesus monkeys were demonstrated by SOGNNAES, SHAW and BOGOROCH (1955). They found that following external administration of I^{131} to the outer surface of teeth isolated from saliva, significant amounts of radioactivity could be detected in the thyroid gland and urine, a fact also demonstrated in the cat by BARTELSTONE (1951). Outward radioisotope movement from the pulp through dentine and enamel was shown to occur with the aid of isotopes I^{131}, P^{32}, K^{42}, Na^{24} and H^{3}. Following systemic administration of these isotopes, significant amounts of radioactivity were detectable in the salivary fluid contained in plastic tubes surrounding the teeth.

It is clear then, from these results, that exchange of substances between external and internal tooth environments can occur. Furthermore, although evidence is lacking, it is possible that such exchanges may be capable of influencing the structural integrity of both the calcified dental tissues and the dental pulp.

Some Pertinent Features of Pulp Histology and their Significance in the Present Investigation.

The histological structure of the human dental pulp has been described by several investigators, including ORBAN (1929, 1944, 1949, 1953, 1957), SCHOUR (1948, 1953, 1960), LANGELAND (1957), SELTZER and BENDER (1965) and OGILVIE and INGLE (1965). The literature, however, contains very few descriptions of the pulp from comparative studies of other species. The histological structure of the fully developed rat pulp has not been described in any detail, although several investigators have utilized the rat pulp as a model to study the histopathologic effects of operative procedures and restorative materials. In fact, the rat has been the most commonly used laboratory animal in most areas of dental research.

It is unnecessary to repeat here in detail all the features

of the human pulp that have been observed in previous studies. However, for the purposes of the present investigation, it is important to mention that a number of histologists have described the presence of a cell-free zone beneath the odontoblasts in the coronal portion of the pulp, the so-called zone of Weil or sub-odontoblastic layer. Adjacent to the cell-free zone is a cell-rich zone consisting mainly of undifferentiated mesenchymal cells (Figs. 1 and 2). According to SCHOUR, the cell-free zone is usually indistinct or absent in the embryonic pulp and at any time when dentine formation is active, while ORBAN states that this zone is only rarely found in young teeth. Since it is not seen in the developing tooth and because its presence varies with the histological treatment, SCOTT and SYMONS (1958, 1961) suggest that the cell-free zone may be an artefact. Most investigators agree that distinct cell-free and cell-dense zones are rarely observed in areas of the pulp of fully formed teeth other than in the subodontoblastic region of the coronal pulp.

Unpublished data on the histology of human pulp tissue by the author has shown that cell-free and cell-dense zones are not artefacts resulting from histological processing of hard and soft tissue in opposition. Whole pulps dissected from pulp chambers

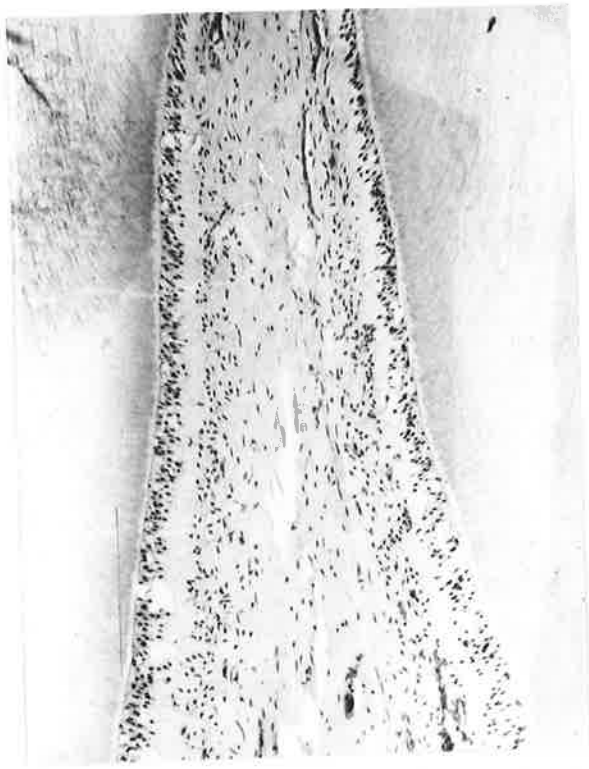


Fig. 1 Coronal pulp canine tooth, patient 12 years, showing distribution of subodontoblastic cell-free and cell-dense zones. Decalcified section. X 180.

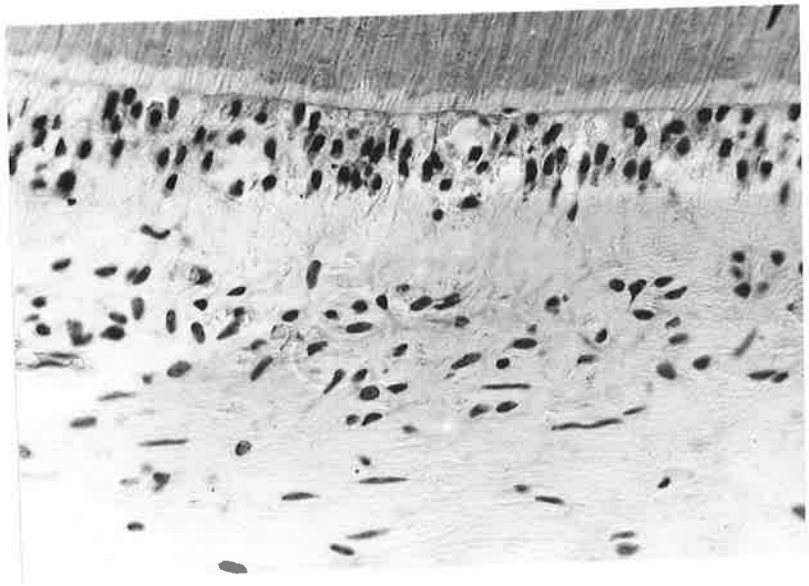


Fig. 2 Coronal pulp of tooth in Fig. 1 showing subodontoblastic cell-free and cell-dense zones. Decalcified section. X 1100.

of erupted teeth following splitting of the teeth show the presence of subodontoblastic cell-free and cell-dense zones in the coronal portion only of the pulp (Figs. 3 and 4); in other words, the appearance is similar to that seen in decalcified sections of teeth. Furthermore, additional unpublished observations by the author on decalcified human material have shown that with routine methods of histological processing, an important factor being rapid and thorough fixation, a common finding is that cell-free and cell-rich zones are, in most cases, found only in certain anatomical regions of the coronal pulp of erupted teeth. These regions are the roof of the pulp chamber, the mesial, distal, lingual and labial or buccal areas, in other words, those internal regions which are linked anatomically to the external crown surface. The base of the coronal pulp (in multi-rooted teeth) and the pulp in the root canals do not exhibit cell-free and cell-dense zones in the subodontoblastic regions.

This point assumes considerable significance particularly in the light of observations made from the animal material used in the present investigation. For example, in the pulp of the erupted rat molar a prominent cell-dense zone lying immediately beneath the odontoblasts can be found in regions corresponding to those

Fig. 3 Coronal pulp of premolar, patient 14 years, dissected from pulp chamber and processed without decalcification, showing presence of cell-free and cell-dense zones in subodontoblastic region. Odontoblast cells not visible since they remain attached to dentinal surface. X 280.

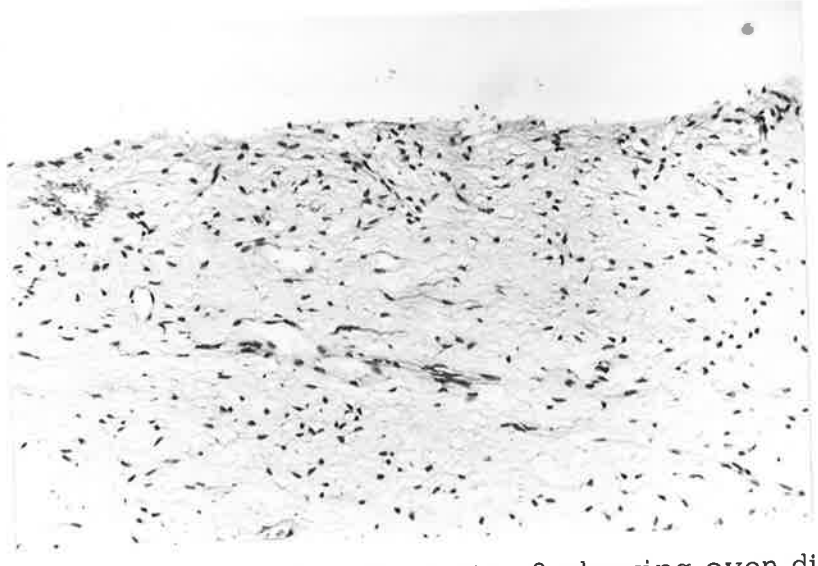


Fig. 4 Root canal pulp of tooth in Fig. 3 showing even distribution of mesenchymal cells. Absence of cell-free and cell-dense zones in subodontoblastic zone. X 280.

in the human pulp which show the presence of a cell-dense zone. The coronal pulps of developing rat teeth do not show such organization in the subodontoblastic region; instead they exhibit an even distribution of mesenchymal cells throughout the whole of the pulp tissue. These features will be considered in greater detail later and will serve as a basis for the suggestion that the cellular organization in the subodontoblastic zone of the coronal pulp in erupted teeth may be mediated by exposure of the tooth to the oral environment.

Dynamic Histology of the Pulp.

Until relatively recently, the dynamic behaviour of the cellular components of the pulp had been considered only in relation to healing following pulpal injury resulting from operative intervention. ZANDER (1953) stated that pulp fibroblasts exhibited a great deal of mitotic activity following operative procedures. He claimed that division of young fibroblasts and undifferentiated mesenchymal cells was the mechanism of odontoblast replacement following death of these cells. According to ZANDER, odontoblasts are incapable of reproducing themselves through mitotic activity. STANLEY (1962) reported the frequent occurrence of mitosis among

mesenchymal pulpal cells following cavity preparation.

Studies on the proliferative potential of cells in fully developed pulps under normal conditions, however, have not received much attention. Data is lacking particularly regarding the mitotic activity of cells in the cell-dense zone from which new odontoblasts are thought to originate. Before reviewing the recent reports relating to the dynamic behaviour of pulp cells, it is essential to trace the development of methods that have been used to study accurately cellular proliferation in other body tissues and organs.

The Dynamic Behaviour of Cell Populations and the Development of Methods Used to Study Cellular Proliferation.

The continual replacement of cells was commented upon in the nineteenth century by PATZELT (1882), BIZZOZERO (1888, 1892) and SHAFFER (1891). These investigators observed intense mitotic activity in the intestinal crypt epithelium and termed these regions "regeneration zones". They claimed that new cells formed in the crypts could move toward the outer surface and become incorporated into the epithelial lining. RAMOND (1904) and JASSINOWSKY

(1925) stated that cells from the intestinal epithelium were continually shed into the intestinal cavity and at the same time were replaced by new cells. In general however, the early histologists did not display much enthusiasm toward a dynamic concept of cell behaviour and quantitative studies were not attempted.

In the last one or two decades the dynamic behaviour of most body tissues has been the subject of detailed investigation and a large amount of information has been obtained. Intense cellular proliferation has been found to occur not only in tissues which show continual growth, for example, hair follicles, ovarian follicles, etc., but also in tissues and organs such as bone marrow, intestinal and respiratory epithelium, spleen and thymus. These body regions do not exhibit any significant alteration in the number or size of their component structures because their cell population is kept relatively constant by a balance between cell production and cell loss. Such organs and tissues have been termed "renewal systems" (LEBLOND and WALKER, 1956).

A variety of techniques have been developed and applied to the study of cell renewal and proliferation. A common approach has been to employ direct visual observations of mitotic

figures and estimate the percentage number of cells undergoing division. One of the limitations of this method is that dividing cells require excellent fixation and staining for their proper identification and quite often this is difficult to achieve. Even when this is attained, it is sometimes difficult to identify mitosis of cells which have small dense nuclei, such as small lymphocytes. Furthermore, when the tissue consists of a composite mixture of cell types, as in the case of bone marrow, it is frequently impossible to ascertain which cell type is undergoing division due to the loss of characteristic cytologic features during the mitotic cycle (LEBLOND, MESSIER and KOPRIWA, 1959).

Other methods that have been used to study cell proliferation involve tagging part of the cell population in some way and then following the increase in number of tagged cells with time or following them as they pass through mitosis. One method of labelling cells is to employ radioactive compounds which become incorporated into certain cell components. In recent years the use of radioactive labels that are specific for nucleic acids in conjunction with radioautography at the cellular level has produced rapid advances in the study of cell proliferation. Several nucleic acid precursors labelled with carbon (C^{14}) and tritium

(H^3) have proved to be very useful in the study of dynamic cell behaviour, but by far the most used substance from this group has been tritiated-thymidine (H^3-thymidine). The H^3-thymidine - radioautography technique is one of two methods employed in the present investigation for the study of cell proliferation and for this reason the development of this technique will now be considered in some detail. This is followed by a similar account of the other method, namely, the use of colchicine.

The Tritiated - Thymidine - Radioautography Technique for the Study of Cellular Proliferation.

Historically, the beginning of radioautography dates back to 1896 when BECQUEREL discovered that crystals of uranium salt placed over a photographic plate produced an image on the developed plate. This image, now termed a radioautograph, was due to the radiation of uranium, unknown at that time. Following recognition of radioactivity by Marie CURIE in 1898, LONDON (1904) exposed a frog to radium emanation. After death, the animal was placed on a photographic plate, and following exposure and development, obtained the first radioautograph of a biological specimen. At about the same time, BOUCHARD, P. CURIE and BALTHAZARD (1904)

were similarly investigating the distribution of inhaled radium emanation in sections of guinea pig tissues. Varying amounts of activity were found in kidney, liver, spleen, heart, brain, lung and adrenal gland, the highest concentrations being in the last two organs.

In 1924 LACASSAGNE and co-workers at the Radium Institute in Paris reported results of their investigations on the distribution of polonium and other elements in histological sections (LEBLOND, 1963). They were responsible for early development of the contact technique of radioautography. The presence of the alpha-ray emitter polonium in rabbit tissues was traced by pressing histological sections of these tissues or the paraffin blocks themselves against photographic plates. Localization of the radioelement was obtained by comparing the stained section with the radioautograph. Although the contact method yielded many useful results, its chief limitations were poor resolution and difficulty in comparing section and photographic plate.

Further advances were required before radioautography could become an accurate histochemical technique. The important step was to devise a method whereby histological section and

photographic emulsion could be combined into one unit by being brought into intimate contact with each other. This was achieved by the so-called coating method devised by BELANGER and LEBLOND (1946). Their technique was to remove emulsion from lantern plates, melt it and spread it on to slides bearing histological sections. Following exposure and development, dark silver grains could be observed directly overlying the sites of the radio-element in the section. Due to the close apposition between section and emulsion, the resolution obtained was very much superior to that achieved by the earlier methods. Further improvement in resolution came from the introduction of the nuclear emulsions which contain a high concentration of very sensitive, uniformly-sized silver bromide crystals. These nuclear emulsions are now available in bulk and are readily melted for coating sections (MESSIER and LEBLOND, 1957). Their use has made it possible to obtain images with high resolution.

Several radioautographic techniques have been developed and of these, apart from the coating method which involves dipping the section-bearing slides in melted emulsion, the only other which has been widely used is the stripping-film technique (PELC, 1947). In this method, the section is covered with a ready-made stripping film previously soaked in water. It does not possess any outstand-

ing advantage over the coating technique, is rather time consuming and tedious; for these reasons, most workers have preferred the former method.

Studies on cellular proliferation involving radioautography have utilized this technique subsequent to the administration of radioactive deoxyribonucleic acid (DNA) precursors into animals. The DNA content of the resting or interphase nucleus remains constant, but shortly before the onset of mitosis a doubling of the cell's DNA content occurs (WATSON and CRICK, 1953). During this period of DNA synthesis the cell takes up various DNA constituents among which are phosphate, the purine bases adenine and guanine and the pyrimidine bases cytosine and thymine (JELLINCK, 1963). If radioactive DNA precursors are injected into an animal they will be incorporated into the nuclei of cells that are about to divide and may be localized subsequently in radioautographs. Such nuclei retain the radioactive label during mitosis and transmit it to the daughter cells.

P^{32}-labelled phosphate was the first nucleic acid label to be used in the study of cellular proliferation by radioautography (LEBLOND, STEVENS and BOGOROCH, 1948). The use of labelled

phosphate, however, had serious limitations because phosphate is taken up by a variety of substances besides DNA: ribonucleic acid (RNA), phospholipids, phosphoproteins and other organic phosphates; a number of these compounds have high turnover rates and therefore P^{32} uptake observed in radioautographs could not be used to assess accurately the sites of DNA synthesis. Furthermore, because of the high energy of beta particles emanating from P^{32} (1.7 MeV maximum), radiation from a radioactive cell nucleus will affect the emulsion at a site far from the cell of origin; consequently, radioautographic resolution with P^{32} is poor.

Adenine labelled with C^{14} has also been used (PELC and HOWARD, 1956; PELC, 1957; WALKER and LEBLOND, 1958). Although C^{14} gives good resolution in radioautographs because of its soft beta emission (0.16 MeV maximum), adenine, like phosphate, is also incorporated into RNA. The detection of labelled DNA requires, therefore, the extraction of RNA from sections, a procedure that is difficult and often incomplete (LEBLOND, MESSIER and KOPRIWA, 1959).

TAYLOR, WOODS and HUGHES (1957) introduced the use of thymidine (the pyrimidine base thymine combined with the pentose

sugar deoxyribose) labelled with tritium. They showed that H^3-thymidine was incorporated into bean root cells during DNA synthesis. Previous to this, REICHARD and ESTBOURN (1951) and FREIDKIN, TILSON and ROBERTS (1956) had shown that thymidine was incorporated efficiently and exclusively into DNA.

HUGHES et al. (1958) extended the H^3-thymidine-radioautography method to animal studies and showed that H^3-thymidine was incorporated exclusively into mouse cells which were synthesizing DNA. A further specific advantage of tritium for radioautography lay in the very high resolution which could be obtained because of the very weak energy (0.018 MeV maximum) and consequent short range of its beta-radiation (FITZGERALD et al., 1951; ROBERTSON and HUGHES, 1958). The activated silver grains resulting from a tritium radioautograph lie largely within 1 to 2 microns of their source, thus giving excellent resolution (Figs. 65 and 66). It is apparent, therefore, that H^3-thymidine is the DNA precursor of choice for accurate determination of the sites of DNA synthesis.

An estimation of the degree of cellular proliferation occurring in a tissue can be determined by administering H^3-thymidine at a level of 1 microcurie per gm. of body weight sacrificing the

animal after 1 hour, preparing radioautographs of that tissue and estimating the percentage number of labelled cells, i.e., the mitotic index.

The Use of Colchicine for the Study of Cellular Proliferation.

The ability of the plant alkaloid colchicine to arrest mitosis was first described by PERNICE in 1899 (EIGSTI, DUSTIN and GAY-WINN, 1949). Following administration of the drug to dogs, PERNICE observed a high number of mitotic figures among the epithelial cells of the stomach and intestine. During the early 1930's, A. P. DUSTIN, Sr., and associates from the University of Brussels carried out extensive studies with colchicine. Results of their work, together with previous experience with other anti-mitotic drugs such as sodium cacodylate, made significant contributions toward establishing the concept of metaphasic arrest by colchicine (EIGSTI and DUSTIN, Jr., 1955).

LUDFORD (1936) showed metaphasic arrest by colchicine of normal and malignant cells in vivo and in vitro. In the same year BRUES reported mitotic arrest of cells by colchicine in regenerating liver. He presented evidence which showed that colchicine stops all nuclear divisions at metaphase, without

affecting the rate at which these divisions occur. SENTEIN (1942 a and b) studied the effect of the drug on larval tissues and in the hypophysis of the guinea pig. He concluded that there was no stimulation of mitosis; colchicine acted merely to block mitotic division at metaphase. The effect of colchicine on various tissues of different animal species was found by SENTEIN (1943 a and b) to be consistently of the same nature. Cells undergoing mitosis were attacked at precisely the same point, namely metaphase, throughout the duration of colchicine action.

Detailed knowledge of the manner whereby colchicine acts to block mitosis at metaphase is not yet completely understood. It is widely accepted, however, that the main action of colchicine, as evidenced by microscopy and by the production of polyploids, is in altering the properties of the spindle (EIGSTI and DUSTIN, 1955). The fibrous and polarized spindle is very rapidly changed into an amorphous "pseudo-spindle" or "hyaline globule" which is incapable of moving the chromosomes (GAULDEN and CARLSON, 1951; INOUE, 1952). As a consequence, the chromosomes remain clumped together in the central part of the cell, thus making the affected cells readily recognizable in histological sections.

LEBLOND and STEVENS (1948) found that 6 hours was the optimal duration for the action of colchicine in studies on cell renewal since up to 6 hours after injection of the drug there were very few anaphases and no telophases. It was concluded that the mitoses had not progressed beyond the metaphase stage. Furthermore, after the 6-hour period, many of the colchicine metaphases were observed to undergo pyknosis and hence could become unrecognizable as such. BULLOUGH (1949) reported that if mouse tissues were examined at time intervals longer than 5 to 6 hours following colchicine administration, a decrease in the number of metaphases occurred; he attributed this to an inhibition of mitosis by colchicine after the 6-hour period. STOREY and LEBLOND (1951), however, commented that this decrease in mitotic numbers may have been apparent rather than real, since it was probable that some colchicine metaphases had undergone pyknosis and fragmentation and therefore become unrecognizable.

The work of BERTALANFFY and LEBLOND (1953) established a number of conditions most suitable for the colchicine technique in cell proliferation studies. In addition to verifying the fact that 6 hours was the ideal time interval between colchicine injection and animal sacrifice, they showed the optimum dose of colchicine for the

rat to be 0.1 milligrams per 100 grams of body weight. This dose of colchicine (administered in a single injection) arrested many metaphases, prevented the accumulation of anaphases and telophases, and did not appear to affect the experimental animals adversely.

A common feature of renewing cell populations is that they exhibit diurnal variations in mitotic activity (COOPER, 1939; BULLOUGH, 1948; BERTALANFFY et al., 1954; HALBERG et al., 1958; MUHLEMANN, EBNETER and RUPF, 1959). For example, BULLOUGH (1948) found that in the dorsal skin and ear epidermis of the adult male mouse the maximum number of mitoses occurred during periods of rest. Variations from day to day, however, did not occur (BULLOUGH, H. F., 1943; BULLOUGH, W. S., 1946, 1948). Therefore, in order to determine accurately the turnover time of a particular cell population it is necessary to calculate the average daily mitotic rate. This can be done by treating 4 or 6 groups of animals at regular intervals over a 24-hour period (STOREY and LEBLOND, 1951; BERTALANFFY and LEBLOND, 1953). In the case of 4 groups of animals, those animals in the first group are injected with colchicine and sacrificed 6 hours later, at which time the second group is injected, and so on. The tissues

of all the animals combined will contain in arrested metaphase all cells which divided during the 24-hour period, and from this data, the daily mitotic rate of the cell population can be calculated. The lag in colchicine action, which may range from 15 to 30 minutes, or even longer in exceptional cases, is not usually taken into account (BERTALANFFY, 1964). If the assumption is made that the total number of cells in a cell population remains constant, then the turnover or generation time of the cell population can be estimated by calculating the time required for 100 per cent of cells within the population to divide (BERTALANFFY, 1964).

The Dynamic Behaviour of Dental Pulp Cells: Review of the Literature.

A few brief reports have appeared on the dynamic behaviour of pulp cells in developing molar teeth studied with H^3-thymidine and radioautography. PAYNTER and HUNT (1961) observed that most of the molar pulpal mitoses in 3-day old rats were confined to the area adjacent to Hertwig's epithelial root sheath with little activity elsewhere. These same workers (HUNT and PAYNTER, 1961) also made similar observations in 2-day old guinea pigs. The molar teeth of hamsters aged from 5 to 30 days were examined

by HOFFMAN and GILLETTE (1962) who found that all of the mitotic activity in the pulp during this period was concentrated in the apical areas adjacent to the root sheath. PAYNTER and HUNT (1964) studied molar tooth development in radioautographs of teeth from animals injected with H^3-thymidine between 18 and 31 days. Their results revealed that in the growing crown mitotic activity of pulp cells was greatest adjacent to proliferating epithelial cells, that is, around the periphery of the crown in the area of the epithelial diaphragm and at the base of the grooves. It was tentatively concluded that the relationship between dentinogenesis and mitotic activity of the inner enamel epithelium may have an important bearing on the determination of the final tooth form.

Only one brief report on the mitotic activity of pulp cells in fully developed molar teeth has appeared. MESSIER and LEBLOND (1960) studying the proliferative potential of rat tissues with H^3-thymidine and radioautography observed only "rare" labelled nuclei in the molar pulp. These workers, however, did not state which cells of the molar pulps were labelled.

The proliferative activity which occurs in the continually-erupting incisor teeth of rodents has been described by several

investigators. SICHER (1942) observed numerous mitoses of fibroblast cells in the region of Hertwig's epithelial root sheath in rats which had been injected with colchicine. "Occasional mitoses" only were noted in areas of the pulp away from the actively growing region. NESS and SMALE (1959) found that 65 per cent of the pulpal mitoses in the untreated rabbit incisor were located in the basal millimetre and adjacent to Hertwig's sheath. The remainder occurred in regions up to 10 millimetres from the base.

MESSIER and LEBLOND (1960) using H^3-thymidine and radioautography observed many labelled nuclei in the root tip of the rat incisor. BLACKWOOD and ROBINS (1964) employing the same technique obtained similar results in the rat incisor. Labelling of pulpal cells was confined to an area bounded by the cervical loop of epithelium, and to the level at which dentine matrix formation commenced. HWANG, TONNA and CRONKITE (1963) studying mouse incisors with H^3-thymidine and radioautography also observed a high labelling index in the preodontoblastic region. Again only a few labelled cells were observed in regions of the pulp other than in the apical area.

MAIN and ADAMS (1966) using both the H^3-thymidine-radioautography and colchicine methods observed many mitoses in the region of Hertwig's sheath. Dividing cells were also seen throughout the rest of the pulp tissue but were much more sparsely distributed. CHIBA (1965) studied the mitotic activity in incisor tooth germs of untreated rats ranging from birth to 76 days of age. Cell divisions in the pulp were found to occur in areas bordering the epithelial tissues, labially and lingually. Mitoses were observed in the central and incisal parts of the pulp but with a lower frequency than in the base. The preodontoblastic layer exhibited dividing cells but cells in the odontoblastic layer in regions where dentine matrix had been deposited were not observed to undergo division.

It is clear then, from these studies, that the greatest degree of mitotic activity in the dental pulp occurs in the apical region of the rodent incisor. In this site both mesenchymal and preodontoblastic cells undergo division. It is also apparent that a lower degree of mitotic division of mesenchymal cells occurs in regions away from the actively proliferating base. However, the precise sites of such activity have not been described. Such information is similarly lacking in the case of the molar pulp.

The Present Investigation.

The present investigation was designed to study the organization and proliferative potential of cells in the dental pulps of neonatal, young and old adult rats. In particular, an important aim of this study was to examine the rat pulp before, during and following tooth eruption into the oral cavity for the presence of structural layers in the subodontoblastic region comparable to the cell-free and cell-dense zones which are found in certain regions of the human pulp.

The first part of the investigation involved a study of the histological structure of the pulp tissues from young and old adult animals and the results are described in Chapter III. Chapter IV deals with the changes in cell distribution which were found to occur in the rat pulp from the period prior to eruption of the teeth to that during and following exposure of the teeth to the oral environment. The results of the investigation into the mitotic activity of pulp cells in adult animals as revealed by the use of H^3-thymidine - radioautography and colchicine are presented in Chapter V. The findings of all these experiments are discussed in Chapter VI where an attempt is made to correlate the present

findings with several previously reported observations and to put forward some suggestions regarding the possible biological significance of the cellular organization in the subodontoblastic zone of the dental pulp.

CHAPTER II

MATERIALS AND METHODS

Animals Used in the Present Study.

Rats of the Hooded-Wistar strain were used exclusively in the present investigation. Except in the foetal and neonatal experiments where animals of both sexes were employed, only male animals were used in the cell proliferation studies. Male animals are preferred in such cases since the mitotic activity of female tissues may be affected by fluctuations during the oestrous cycle (BULLOUGH, 1950; EBLING, 1954). The animals were housed in a well-ventilated room maintained at a constant temperature. They were fed a normal diet (see appendix ii) and water ad libitum.

The dentition of the rat is monophyodont and is comprised of one incisor and three molars in each half of upper and lower jaws. The incisor is situated anterior to the molars in the maxilla while in the mandible it extends below and beyond the molars (Figs. 5 and 6). The incisors do not have a root portion but consist of a large, elongated crown, the labial surface of which is covered by enamel and the lingual by cementum. The molar crowns, with the exception of the cusp tips which show exposed dentine, have a complete enamel covering, while their roots are

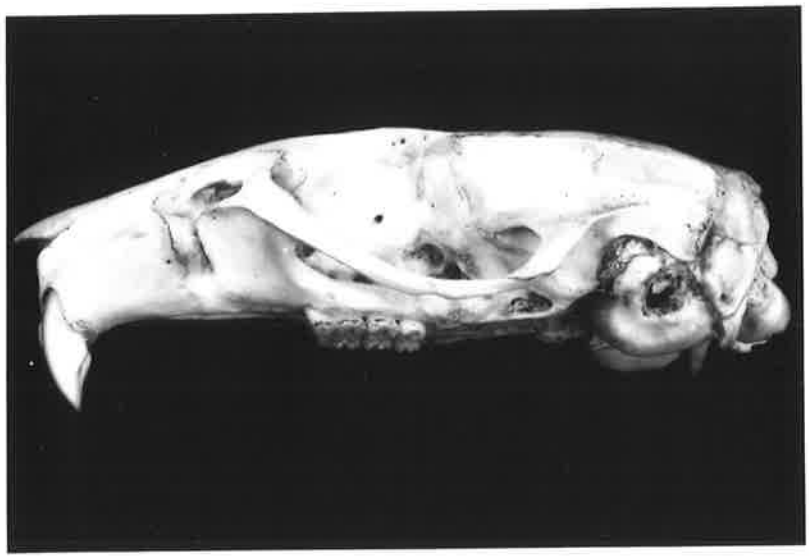


Fig. 5 Adult Rat maxilla, approx. 3 X actual size.

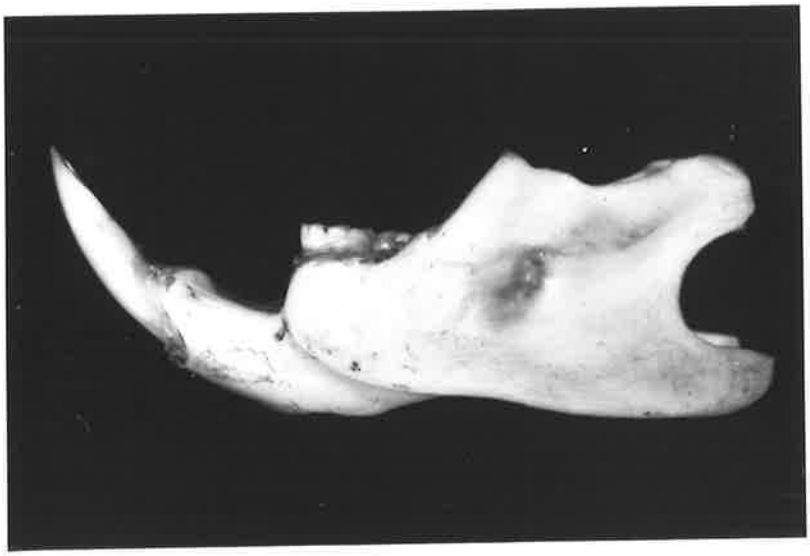


Fig. 6 Adult Rat mandible, approx. 3 X actual size.

covered by cementum.

The incisors exhibit continual apical growth and eruption throughout the animal's life and show considerable attrition at their incisal edges. Continual eruption does not occur in the molars and they are abraded at a slow rate.

The incisor teeth of the animals used in the present investigation exhibited varying degrees of attrition. The molar teeth of the young animals (i.e. those up to 8 weeks of age) showed no signs of attrition. The older animals (6 to 7 months) had only moderate abrasion of their occlusal surfaces. Incisor and molar teeth from all animals, however, were entirely free from erosion and dental caries. Thus, the pulp tissues under study had been subjected to minimal external irritation and could therefore be considered normal. On the other hand, many animals, particularly the older ones showed evidence of periodontal disease. A common histological observation was the presence of impacted hair and food debris in the region of the interdental papillae with concomitant acute and chronic inflammatory lesions (Fig. 7).

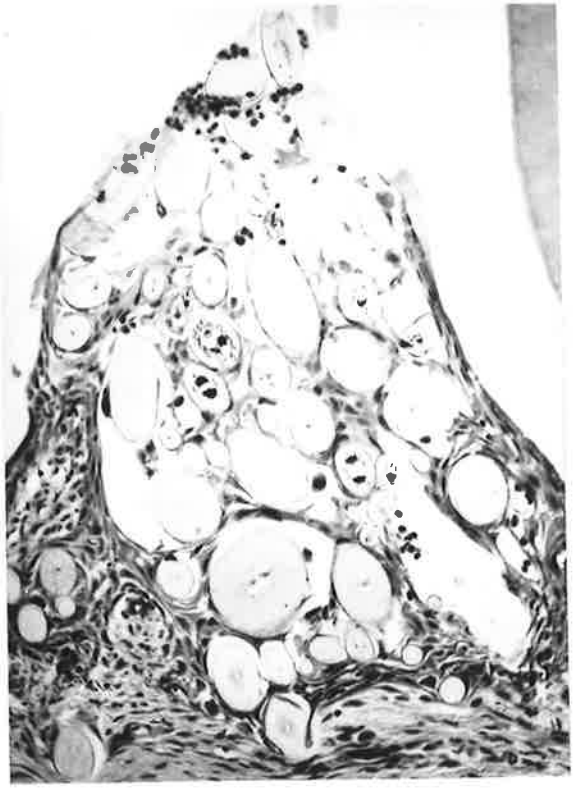


Fig. 7 Interdental papilla region, 6 month rat, showing impacted hair and food debris. X 800.

Techniques Used to Study the Histological Structure of the Incisor and Molar Pulps from Adult Animals.

For the study of incisor and molar pulps in decalcified sections of teeth, the upper and lower jaws were placed in Bouin's fixative (appendix iii) immediately after sacrifice (by decapitation under ether anaesthesia). Following a 12 to 24 - hour fixation period, the jaws were washed in 70 per cent alcohol for one hour and then placed in 30 per cent formic formate decalcifying fluid (appendix iii). The specimens were kept at 37^{o}C and were continually agitated on a mechanical vibrator; the decalcifying fluid was changed every 2 days and completion of decalcification was determined radiographically. The individual incisors and each molar quadrant from upper and lower jaws were dissected out and placed in 5 per cent sodium sulphate for 8 hours to neutralize any excess acid. The specimens were then dehydrated in alcohols of ascending strength, cleared in methyl salicylate and embedded in paraffin wax under vacuum. Longitudinal and transverse sections of the incisors and mesio-distal and bucco-lingual sections of the molars were cut on a Spencer 820 microtome, stained with Ehrlich's haematoxylin and counterstained with eosin.

Histological sections of incisor pulps that had not been subjected to decalcification were also prepared. Pulps were removed from lower incisor teeth in the following manner: a high-speed dental handpiece with water-spray was used to cut two longitudinal grooves in the tooth which was then split by gentle leverage applied with a fine straight chisel. By this method, it was possible to dissect away from the dentine practically the whole incisor pulp in a relatively intact form (Fig. 8). Pulps removed in this manner were fixed in either Bouin's fluid, 10 per cent formol-saline or Zenker's solution. After washing, the pulps were dehydrated in ascending strengths of alcohol, cleared in benzene and vacuum embedded in paraffin wax. Transverse and longitudinal sections of pulps processed in this manner were cut at 3 to 4 microns and stained with haematoxylin and eosin.

Photomicrographs of representative decalcified and undecalcified pulp tissues were taken with an Olympus PM 6 camera attached to an Olympus E C E Tr microscope using Adox K B 14 film and were printed on Ilford bromide paper.

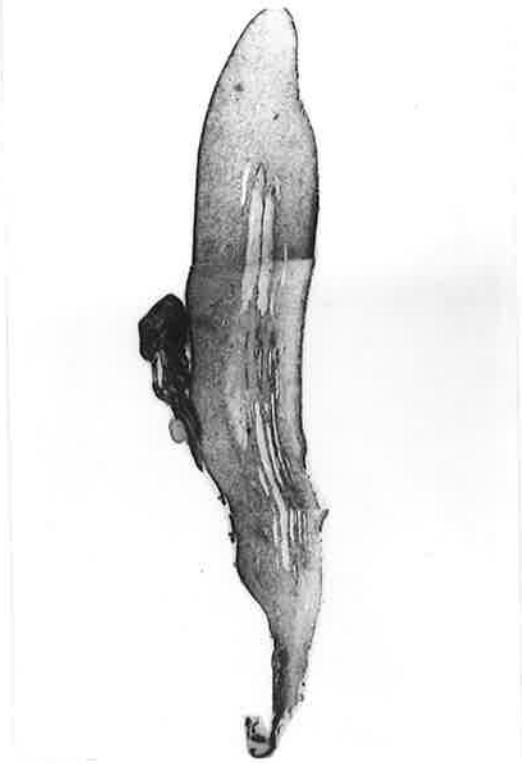


Fig. 8 Lower incisor pulp from 6 month old rat dissected from pulp chamber and processed without decalcification. Longitudinal section. X 10.

Histological Study of the Development of the Subodontoblastic Cell - Dense Zone.

In order to determine the period during which the dense layer of mesenchymal cells forms beneath the odontoblasts in the coronal part of the molar pulp, sections of the maxillary and mandibular molars were prepared from normal, untreated animals at the following ages: 4, 8, 12, 16, 18, 20, 21, 22, 24, 25, 30, 35, 40 and 45 days.

An Investigation into a Possible Mode of Formation of the Subodontoblastic Cell - Dense Zone using Colchicine.

Having established the time at which the subodontoblastic cell-dense layer forms, an experiment was then carried out to determine whether the increased number of cells in this region results from mitosis of the mesenchymal cells lying beneath the odontoblasts.

Pairs of animals of both sexes aged 17, 18, 19, 20, 21, 22, 23, 24, 25 and 28 days were given a single subcutaneous dose of colchicine, 0.1 mg. per 100 gm. body weight. The animals were sacrificed 4 hours after colchicine administration and the maxillae and mandibles were prepared for histological examination.

Mitotic Activity of Dental Pulp Cells in Young and Old Adult Animals Studied with Colchicine.

Sixteen male rats, 6 to 8 weeks in age and weighing between 90 and 110 grams were randomly assigned to one of 4 groups, 4 animals per group. A dose of colchicine ($C_{22}H_{25}O_6N$, British Drug Houses Ltd., B.D.H. Laboratory Chemicals Division, Poole, England.) 0.1 mg. per 100 gm. of body weight was administered subcutaneously in the abdomen of each animal 6 hours prior to sacrifice. To detect any diurnal variation in mitotic activity, the animals of Group 1 were injected at 12 noon and killed at 6 p.m.; Group 2 injected at 6 p.m. and killed at 12 p.m.; Group 3 injected at 12 p.m. and killed at 6 a.m. on the following day; Group 4 injected at 6 a.m. and killed at 12 noon, thus making up a complete 24-hour period. To determine any difference in mitotic activity due to age of the animal, the same experiment was repeated using animals aged 6 to 7 months and weighing 300 to 350 gm.

The incisor and molar teeth from upper and lower jaws of each animal were processed for histological examination in the manner described previously. Sections were examined under oil

immersion (1000 X magnification) for the presence of dividing pulp cells that had been arrested in the metaphase stage of mitosis by the action of colchicine. All regions of the incisor pulps and the coronal and root canal regions of the molar pulps were examined in this manner. Sections of tongue epithelium from each animal were also examined to check the success of colchicine administration.

Mitotic Activity of Dental Pulp Cells in Young Adult Animals Studied with Tritiated -Thymidine and Radioautography.

Twelve male rats approximately 8 weeks of age and weighing between 100 and 110 gm. were randomly assigned to one of 4 groups, 3 animals per group. Each animal received two intraperitoneal doses, 1 microcurie per gm. body weight of H^3-thymidine (specific activity 5.0 curies per millimole; TRA-61 Thymidine -6 -T (n), The Radiochemical Centre, Amersham, Buckinghamshire, England). The first dose was given 6 hours prior and the second 3 hours prior to sacrifice. Animals of Group 1 were injected at 12 noon and 3 p.m. and sacrificed at 6 p.m.; Group 2 injected at 6 p.m. and 9 p.m. and sacrificed at 12 p.m.; Group 3 injected at 12 p.m. and 3 a.m. and sacrificed at 6 a.m.; Group 4 injected at 6 a.m. and 9 a.m. and sacrificed at 12 noon. Immediately after

sacrifice the upper and lower jaws from each animal were fixed in Bouin. The incisor and molar teeth were decalcified, dehydrated, cleared and embedded in the manner described previously. Tongue tissue from each animal was also processed to check the success of the labelling procedure.

Sections were cut at 4 microns and mounted on glass slides which had been cleaned with chromic acid, washed in distilled water, dried, dipped into a solution containing 0.5 per cent Davis Bacteriological Gelatin and 1:20,000 merthiolate in distilled water, and allowed to dry. The section-bearing slides were passed through xylol, absolute, 90, 70 and 50 per cent alcohols and finally into distilled water.

In a darkroom illuminated only by an Ilford F safelight (No. 904), the slides were coated with Ilford K-2 nuclear emulsion (Ilford Limited, Essex, England). The emulsion was prepared in the manner suggested by CARO and VAN TUBERGEN (1962) and the slides coated with emulsion by a modification of the dipping method of MESSIER and LEBLOND (1957). In the preparation of the emulsion, 20 gm of K-2 gel were placed into 20 ml. of distilled water in a Coplin jar and melted at 45^{o}C for 15 minutes. After

gentle stirring, the emulsion was allowed to cool to 23°C. The slides were grasped at their labelled end with artery forceps, dipped into the emulsion for 1-2 seconds and withdrawn with a slow uniform motion. The excess emulsion was drained and the slides allowed to dry in a vertical position. When the emulsion had dried the slides were placed in open plastic slide boxes which in turn were placed inside a light- and air-tight tin. The slides were maintained throughout the exposure period under conditions as suggested by Professor D. A. CAMERON (personal communication), namely, at room temperature, in an inert atmosphere of nitrogen gas and containing a small amount of solid desiccant (magnesium perchlorate).

After a suitable exposure period which ranged from 4 to 8 weeks, the slides were developed for 6 minutes in Kodak D-19b developer, placed in 1 per cent acetic acid for 10 seconds, fixed for 5 minutes in Ilford rapid fixer, washed for 20 minutes in running tap water and then in 3 changes of distilled water. They were then stained with haematoxylin and eosin, dehydrated in alcohol and mounted. All regions of the incisor and molar pulps were examined under oil immersion for the presence of labelled cells.

Due to the possibility of producing artefacts in radioautographs by a variety of causes, for example, extraneous light during exposure, film scratches, dust and debris, external penetrating radiation, etc., the procedure suggested by COMAR (1955) was followed; namely, a control experiment was carried out with identical tissues which did not contain any H^3-thymidine. Rats of comparable age and weight to those used in the experimental series and which had not been subjected to isotope administration were sacrificed and histological sections of tongue, epithelium and teeth prepared. Following this the sections were carried through the normal radioautographic procedure, that is, coating with emulsion, exposure, development and fixation. They were stained and examined microscopically to detect any darkened areas which might otherwise be attributed erroneously to the effects of the isotope in the tissues.

Tritiated - Thymidine Administration to Foetal and Neonatal Animals to Study the Mitotic Potential of Pulp Cells Over a Long Period of Time.

In this experiment 18 animals were given daily intraperitoneal injections of H^3-thymidine, 1 microcurie per gm body

weight, from the period of birth to 10 to 13 days. Some of these animals had, in addition, been injected with 2 microcuries of H^3-thymidine at approximately 19 to 20 days in utero. Access to these animals had been obtained via the Caesarean approach. The animals were sacrificed at about 6 months of age and radio-autographs of molar and incisor teeth prepared and examined in the manner described previously.

CHAPTER III

HISTOLOGICAL STRUCTURE OF THE ADULT RAT DENTAL PULP

INCISOR PULP

General

The pulp chamber of the continually-growing incisor tooth occupies almost the total cross-sectional area of the tooth at the apical end and tapers uniformly to a narrow channel in the incisal region (Figs. 9 and 10). The incisor pulp is an extremely vascular tissue, containing several large arteries in its central core and smaller ones at the periphery (Figs. 9 and 11). In addition to numerous blood vessels, the pulp contains nerves, fibres, and cells of the reticulo-endothelial or macrophage system which are situated mainly in close proximity to blood vessels. The most numerous cellular components of the pulp are the relatively undifferentiated mesenchymal cells and the specialized odontoblasts.

The process of active growth and differentiation of mesenchymal into odontoblast cells occurs at the base of the tooth. In this region the density of mesenchymal cells is very great, particularly in the region immediately adjacent to the labial and lingual dental papillae (Figs. 12 and 13). The newly-formed odontoblasts and those mesenchymal cells which have not undergone differentiation in the basal region continually migrate from

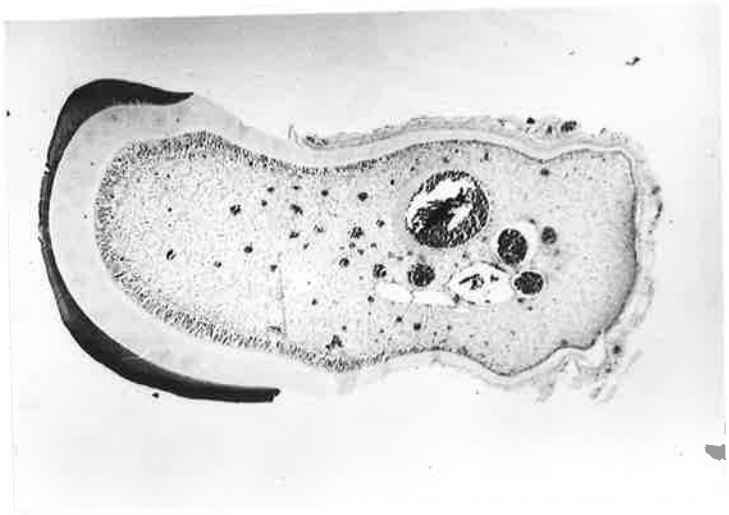


Fig. 9 Base of incisor pulp; pulp tissue occupies almost total cross-sectional area. X 80.

Fig. 10 Incisal tip region showing narrow cross-sectional area of pulp chamber. X 80.

Fig. 11 Pulp of maxillary incisor showing great vascularity of this tissue. X 110.

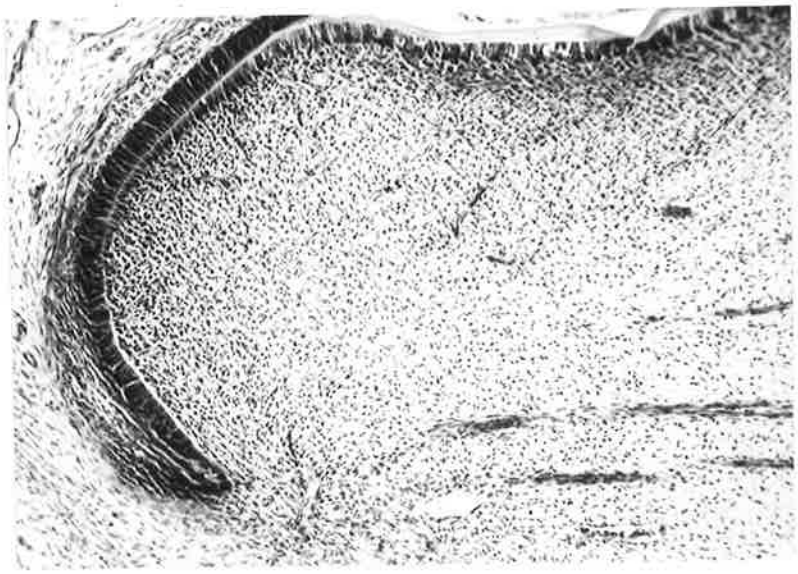


Fig. 12 Base of maxillary incisor pulp, labial aspect. X 200

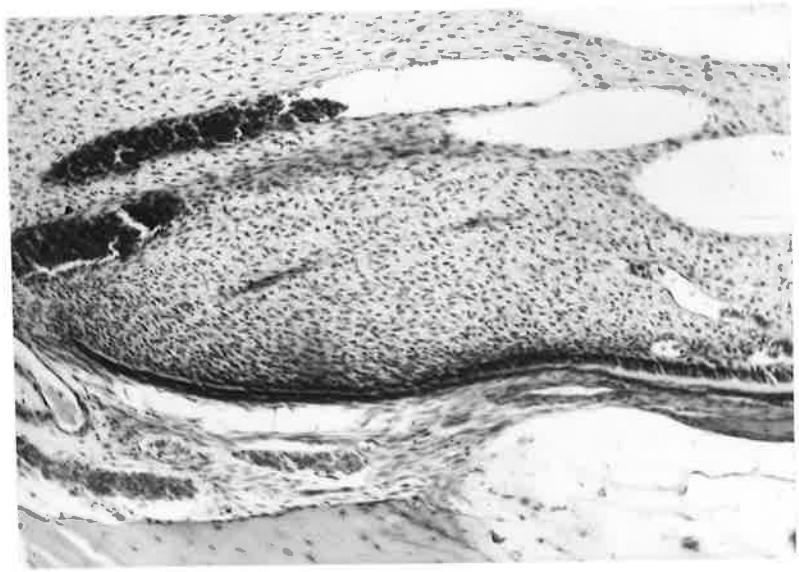


Fig. 13 Base of maxillary incisor pulp, lingual aspect. X 280.

the base toward the incisal part of the pulp. In this way, the pulp, dentine, cementum and enamel tissue which has been lost through attrition in the incisal region is replaced and the bulk of tooth tissue is maintained relatively constant. This forward movement of pulp cells will be referred to again later for it explains the characteristic arrangement of mesenchymal cells in the subodontoblastic zone of the incisor pulp.

The arrangement, morphology and interrelationship of odontoblast and mesenchymal cells is best seen in sections of pulp tissue which has been dissected away from the dentine following splitting of the tooth and placed immediately in fixative solution. By this technique it is possible to prepare histological sections of pulp tissue which are superior to those obtained from decalcified material by virtue of more rapid and thorough fixation and elimination of the necessity for exposing the tissue to decalcifying agents.

Odontoblast Cells

A significant feature of pulps removed in this manner is that a large number of intact odontoblast cells are detached from the dentine and retain their normal relationship to the remainder

of the pulp tissue (Figs. 14, 15 and 16). Frequently, however, the cell bodies of the odontoblasts are severed and only the cell nuclei remain attached to the pulp tissue (Figs. 14 and 17). This situation is in contrast to that occurring in the human tooth where, in the experience of the author, considerable difficulty has been experienced in removing even a few isolated clumps of odontoblasts with the pulp tissue following splitting of the tooth. In such cases, practically all the odontoblasts remain attached to the dentine surface. Fig. 3 shows the periphery of the coronal portion of pulp removed from a human premolar following splitting of the tooth, while Fig. 18 shows the odontoblasts remaining attached to the dentinal tubules. This observation on the human tooth has, as mentioned earlier, been reported previously by PINCUS (1950, 1952, 1953) and ZERLOTTI (1964).

Odontoblasts from apical, middle and incisal regions of the pulp show close uniformity in morphology, being tall columnar cells with an oval, darkly staining nucleus situated at the basal end of the cell (Fig. 16). The dense network of capillaries in the odontoblastic layer which is clearly evident in decalcified sections is also readily seen in pulps detached from the dentine (Fig. 16).

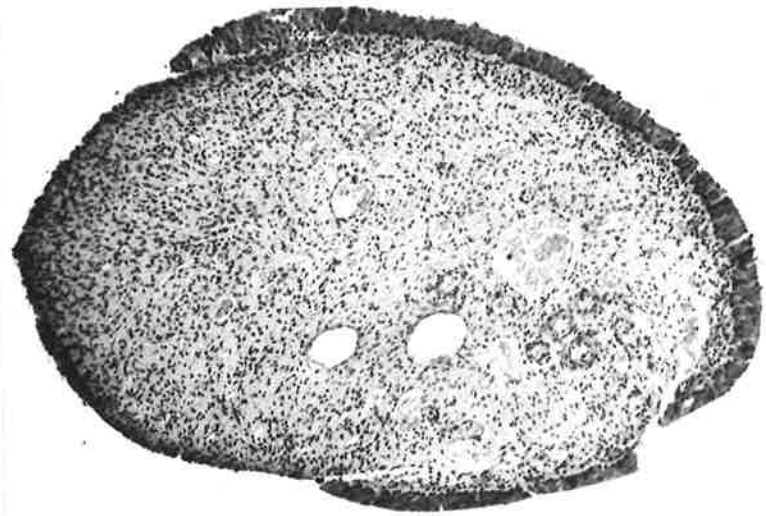


Fig. 14 Lower incisor pulp dissected from pulp chamber, processed without decalcification, cut transversely showing areas of intact and severed odontoblasts. X 170.

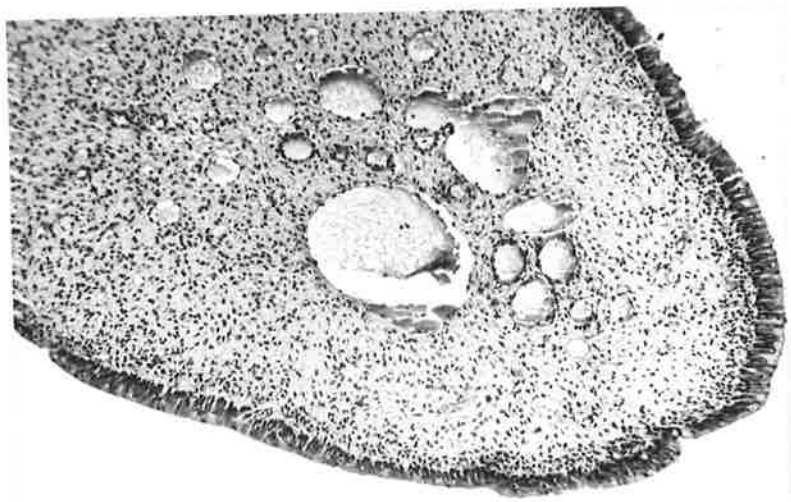


Fig. 15 Lower incisor pulp processed without decalcification showing layer of intact odontoblasts. X 280.

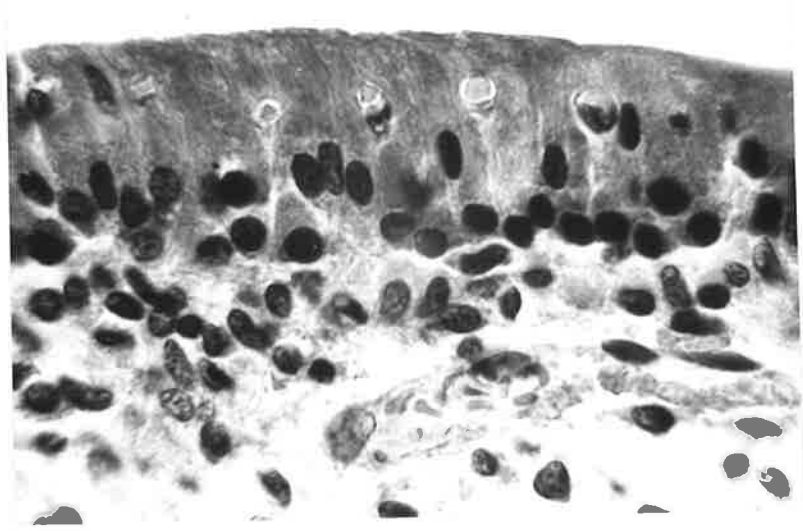


Fig. 16 Intact odontoblasts from lower incisor pulp shown in Fig. 15; capillaries evident between odontoblast cells. X 2000.

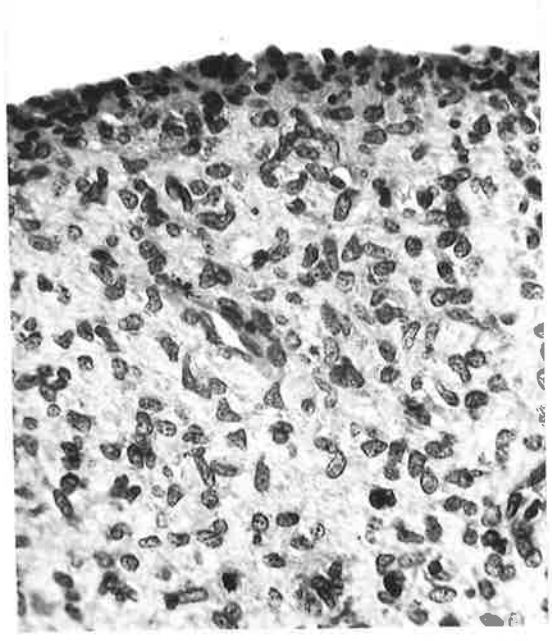


Fig. 17 Lower incisor pulp shown in Fig. 14; cell nuclei of severed odontoblasts line periphery. X 800.

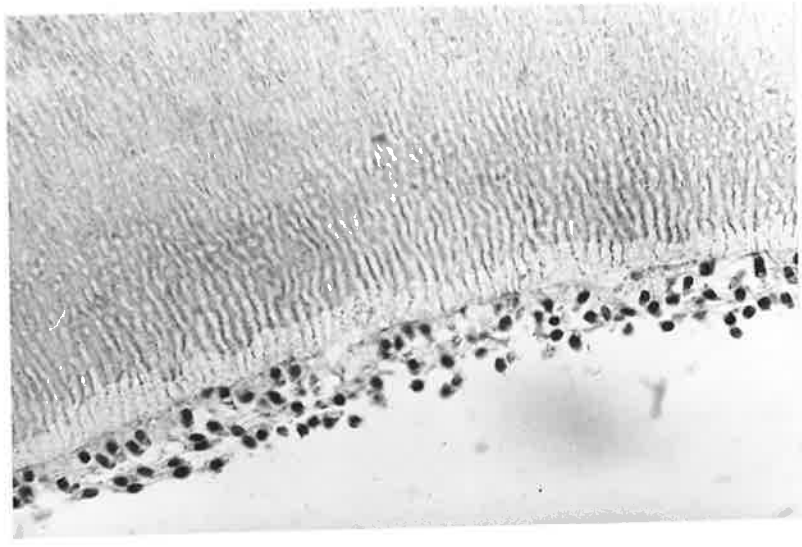


Fig. 18 Severed odontoblast cells remaining attached to dentine surface following splitting of human premolar tooth and dissection of pulp tissue. X800

Mesenchymal Cells

The mesenchymal cells are by far the most numerous cellular elements of the pulp. These cells are almost uniform in size although their shape varies somewhat (Fig. 19). Their number does not appear to decrease significantly with increasing age of the animal.

At the periphery of the pulp in the subodontoblastic zone, mesenchymal cells lie in close proximity to the odontoblasts (Figs. 20, 21 and 22). There is no distinct cell-free zone beneath the odontoblasts comparable to the Zone of Weil which exists in the coronal pulp of human teeth. The mesenchymal cells lying adjacent to the odontoblasts are more densely packed than in the more central parts of the pulp. This subodontoblastic cell-dense zone is seen in all regions of the incisor pulp - from the actively proliferating base to the incisal region (Figs. 12, 13, 20, 21 and 22). It tends to be more prominent on the labial than on the lingual aspect (Fig. 11). The cell-dense zone is not an artefact resulting from histological processing of soft and calcified tissue in apposition since the appearance is the same in decalcified

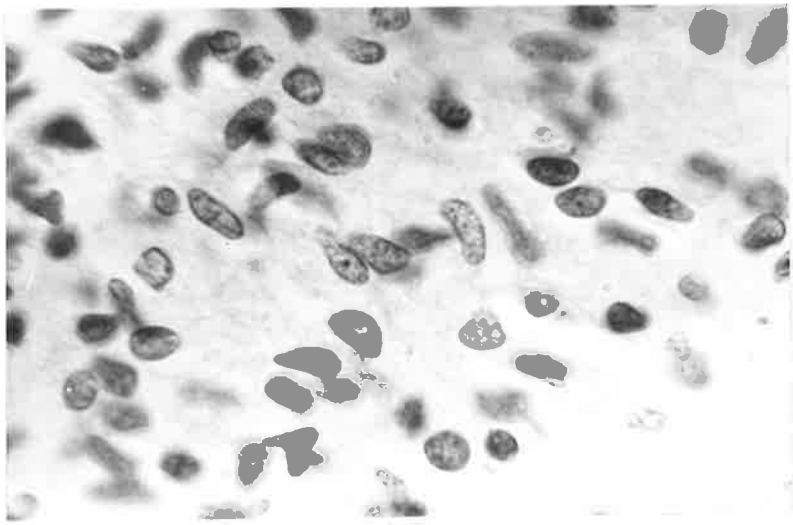


Fig. 19 Mesenchymal cells of incisor pulp. X 2000.

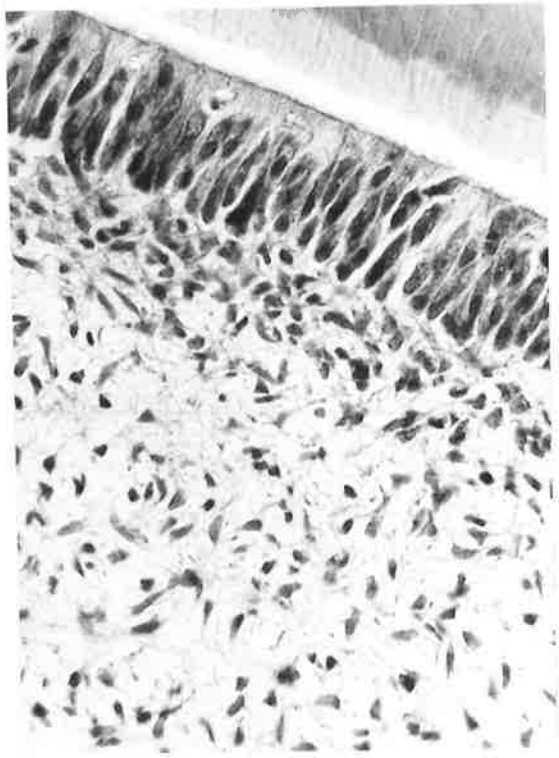


Fig. 20 Odontoblast cell layer, subodontoblastic mesenchymal cell-dense zone and remainder of mesenchymal pulp cells in mid-region of incisor pulp.
Decalcified section. X 800.

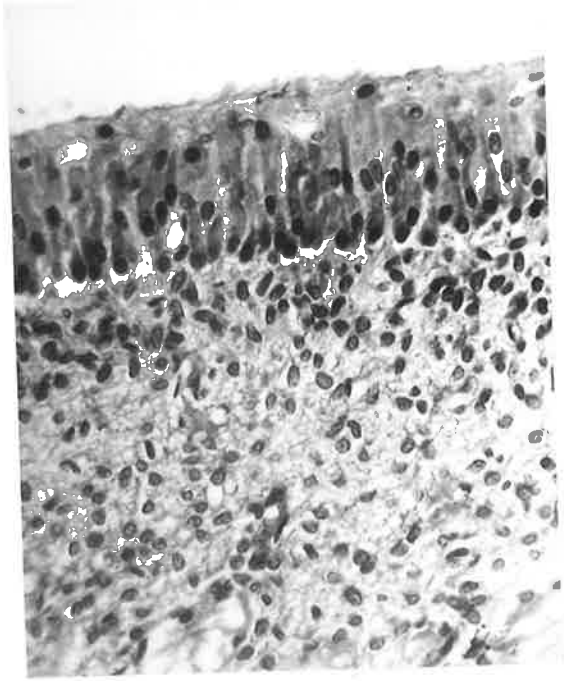


Fig. 21 Odontoblast layer, subodontoblastic cell-dense zone and remainder of pulp tissue in mid-region of incisor pulp processed without decalcification. X 800.

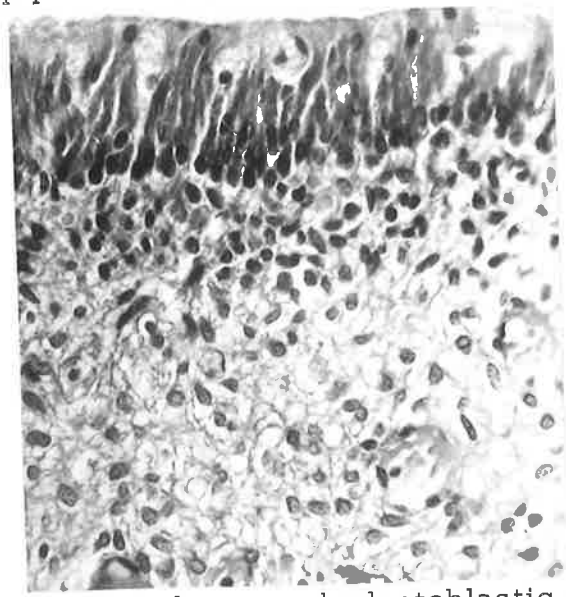


Fig. 22 Odontoblast layer, subodontoblastic cell-dense zone and remainder of pulp tissue in incisal region of incisor pulp processed without decalcification. X 800.

and undecalcified specimens (Figs. 20, 21 and 22). The development of this dense layer of cells is discussed in Chapter IV.

MOLAR PULP

General

The molar pulp consists of a coronal portion whose shape corresponds closely to the external morphology of the tooth crown and a radicular portion consisting of fine canals of tissue extending along the root lengths and emerging at or near their apices.

The histological structure of the molar pulp is basically similar to the incisor pulp in that it contains blood vessels, nerves, intercellular substance, cells of the macrophage system, mesenchymal and odontoblast cells. However, a wider variation exists in the morphology and arrangement of cells in the molar pulp over that found in the incisor pulp.

Owing to the exceedingly small size of the rat molar tooth, the pulp cannot be removed by tooth splitting and subsequent dissection. The histological features of the molar pulp must, therefore, be observed in decalcified sections of the teeth.

Variation in Odontoblast Morphology

A feature of the rat molar pulp which is also seen in the human pulp is the variation existing in the form and arrangement of odontoblast cells in different regions of the pulp. In the roof and lateral aspects of the coronal pulp in young animals, odontoblasts are narrow, elongated cells with correspondingly-shaped nuclei occupying about half of the cell volume (Figs. 23 and 24). The cytoplasm tapers off near the predentine to form the odontoblastic process. Numerous capillaries are seen between these cells (Figs. 23, 24 and 34).

In the narrow pulpal horns the odontoblasts are much smaller in size, being extremely flattened, more densely packed and having few capillaries between them (Figs. 25 and 26). Similarly, odontoblasts on the floor of the coronal pulp tend to be smaller and have less cytoplasm (Fig. 27). In the narrow root canals the situation resembles that in the pulpal horns. Odontoblasts are considerably flattened and are recognized as such only by their cytoplasmic extensions into the dentine. Some areas of the root canals may show complete absence of odontoblasts (Fig. 28).

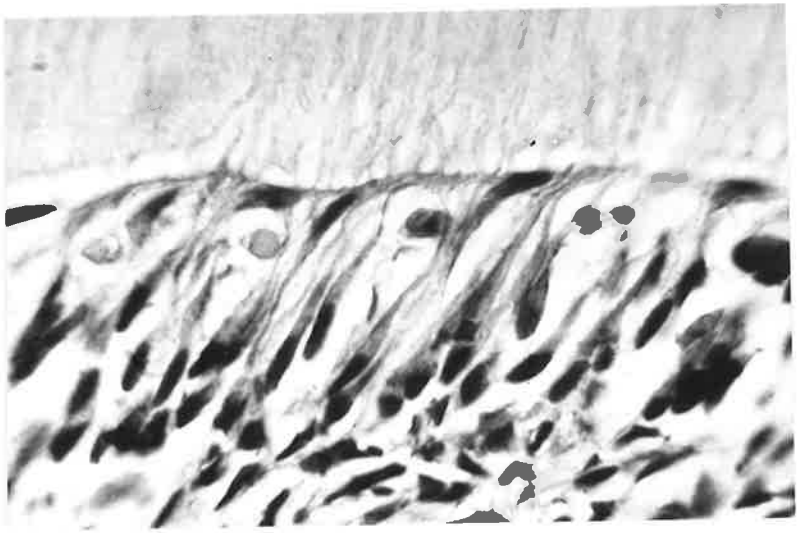


Fig. 23 Odontoblast cells in roof of pulp chamber of molar tooth, young adult rat. X 2000.

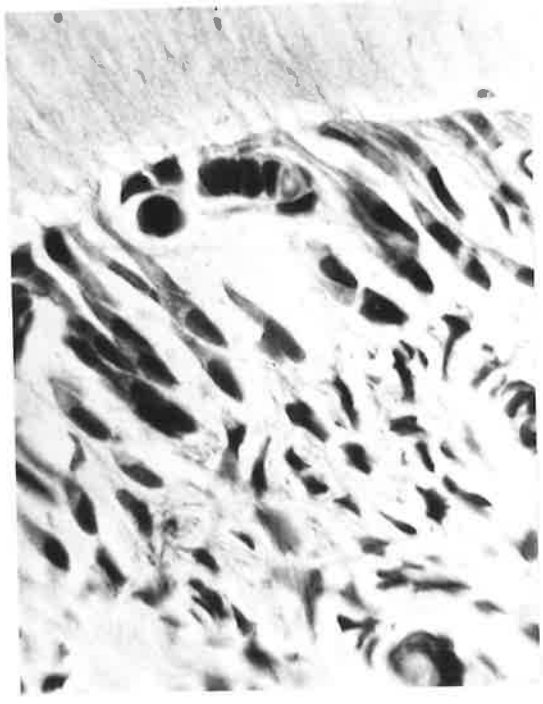
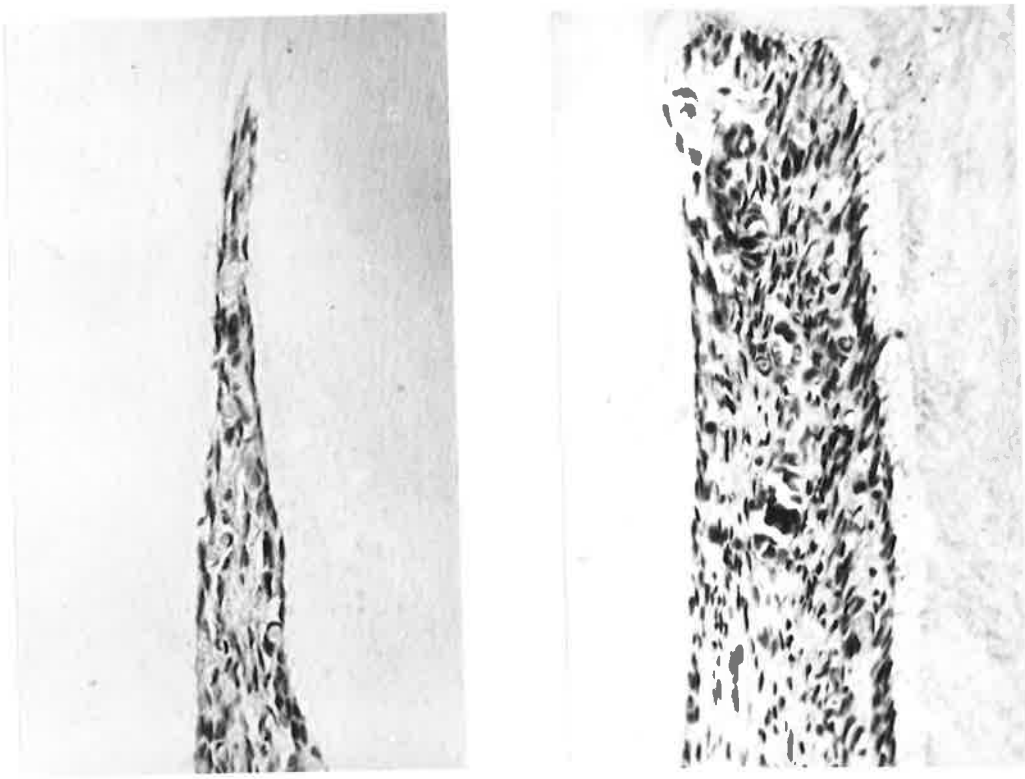


Fig. 24 Large capillary between odontoblast cells in roof of pulp chamber of molar tooth. X 2000.



Figs. 25, 26 Pulp horns of molar teeth young adult rats showing flattened odontoblast cells. X 800.

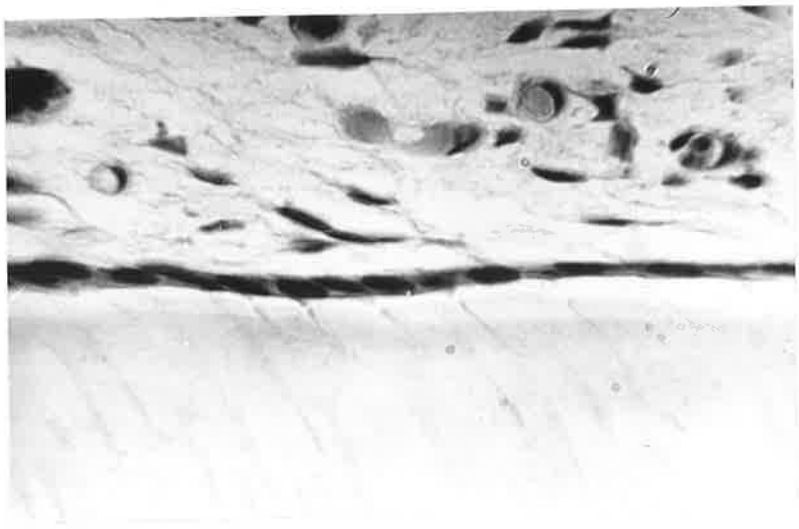


Fig. 27 Base of coronal pulp, molar tooth young adult rat. Odontoblast nuclei occupy large part of cell body. X 2000.

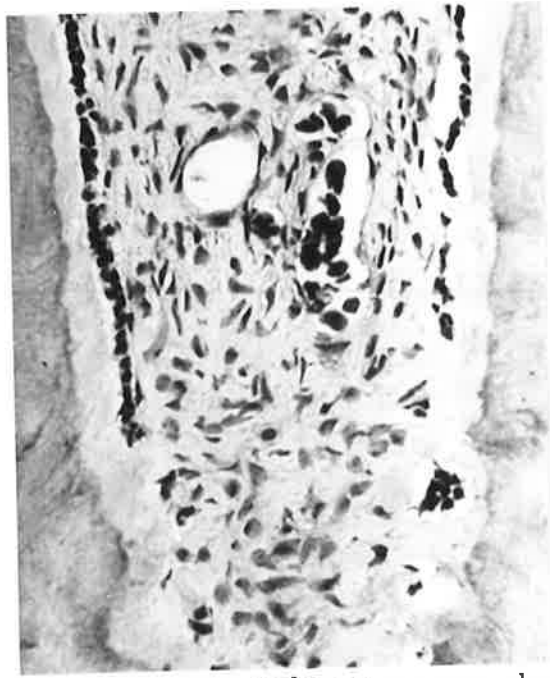


Fig. 28 Area of root canal pulp near apex showing absence of odontoblast cells. X 800.

The pulps of older animals (6 to 7 months of age) also exhibit a variation in odontoblast morphology in different regions of the pulp. A notable age change, however, is that odontoblasts in the roof and lateral aspects of the coronal pulp of older animals are smaller, have proportionately larger and more rounded nuclei and less cytoplasm than corresponding cells in younger animals (cf. Figs. 23 and 29). The vascularity of the molar pulp appears to decrease markedly with age, particularly within the odontoblast cell layer. In contrast to the situation found in young tissue, very few capillaries are to be found between odontoblasts in the pulp tissue of animals aged 6 to 7 months.

Subodontoblastic Zone

As in the case of the incisor pulp there is no cell-free zone (Zone of Weil) immediately beneath the odontoblasts in the coronal pulp. Instead, a mesenchymal cell-dense subodontoblastic zone is present, but only in certain areas of the coronal pulp. The subodontoblastic cell-dense zone is confined to the roof and lateral aspects (i.e. mesial, distal, buccal and lingual) of the coronal pulp (Figs. 30, 31, 32, 33, 34, 35, 36, 37 and 38). This arrangement of mesenchymal cells is not observed in the base

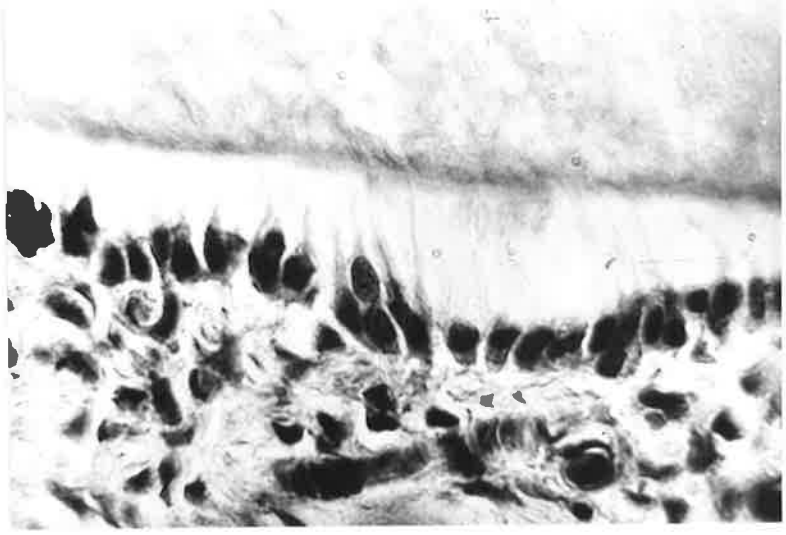


Fig. 29 Odontoblast cells in roof of pulp chamber of 6-7 month old rat. Nuclei occupy almost total cell volume. X 2000.

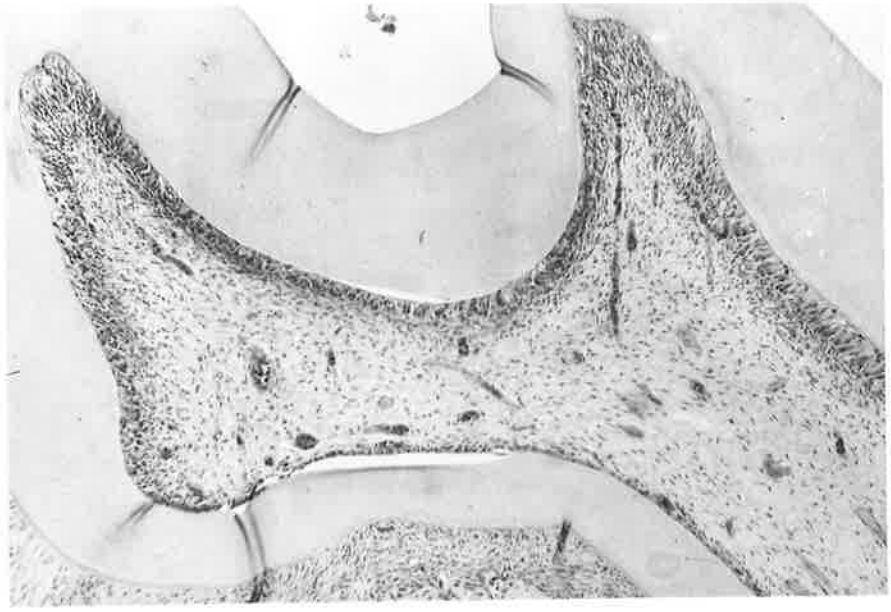


Fig. 30 Mesio-distal section 3rd. molar, young adult rat showing selective distribution of subodontoblastic cell-dense zone in coronal pulp. X 190.

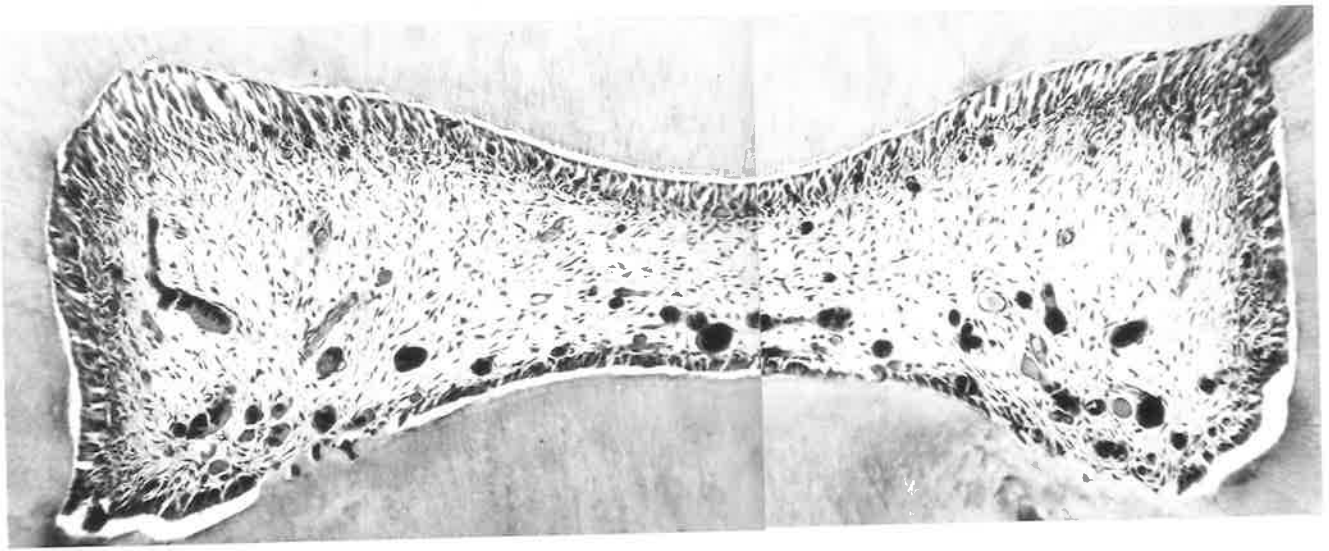


Fig. 31 Bucco-lingual section 1st. molar young adult rat showing selective distribution of subodontoblastic cell-dense zone in coronal pulp. X 300.

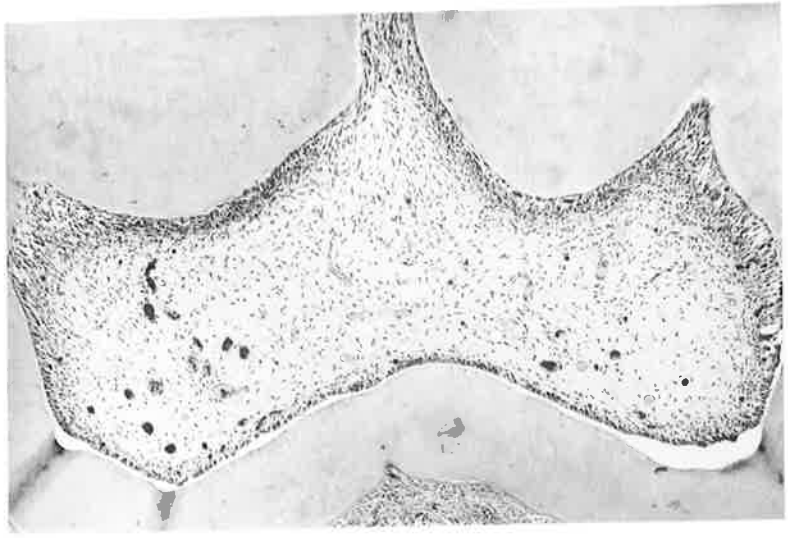


Fig. 32 Mesio-distal section 2nd. molar young adult rat showing selective distribution of subodontoblastic cell-dense zone in coronal pulp. X 170.

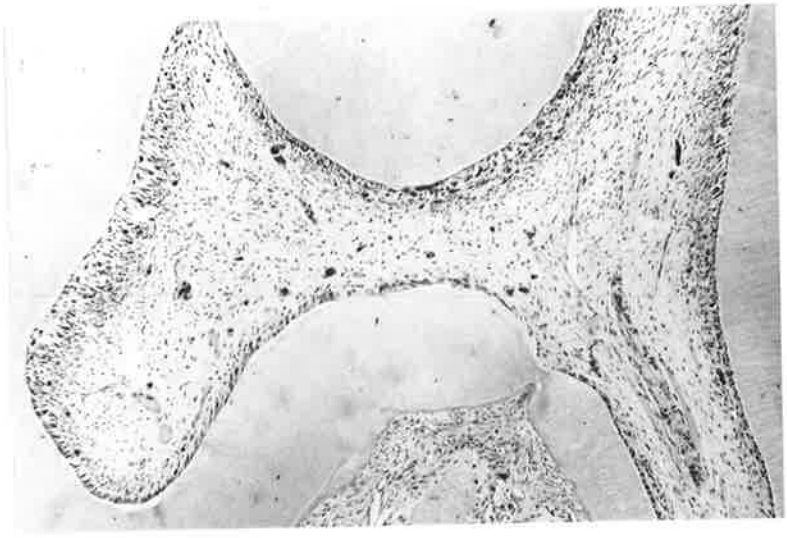


Fig. 33 Mesio-distal section 2nd. molar young adult rat showing presence of subodontoblastic cell-dense zone in roof and lateral aspects of pulp chamber and absence of such a zone in base of pulp and in the root canals. X 170.

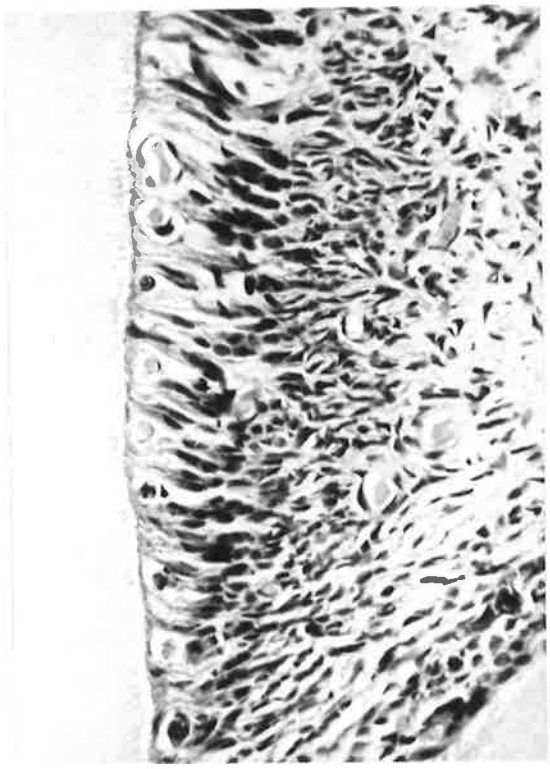


Fig. 34 Mesial aspect, coronal pulp 1st. molar young adult rat showing odontoblastic cell layer, and subodontoblastic cell-dense zone. Numerous capillaries evident between odontoblasts. X 800.

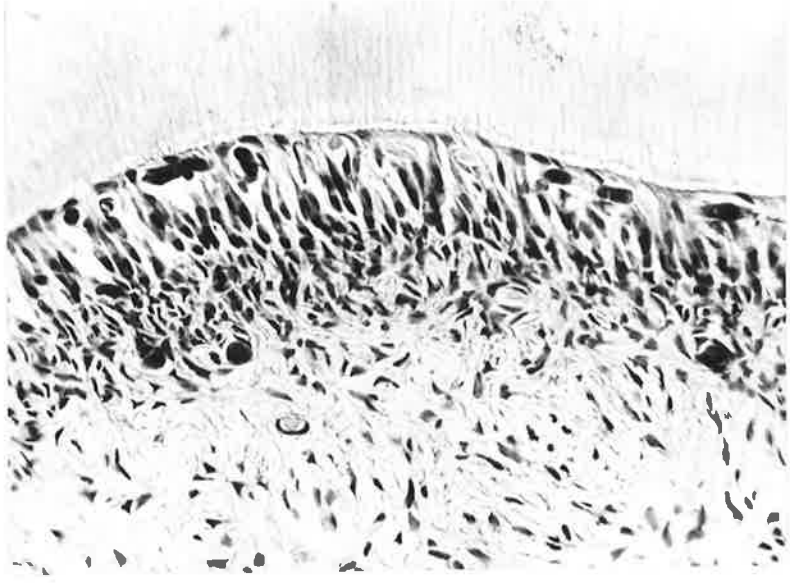


Fig. 35 Roof of pulp chamber 2nd. molar, young adult rat showing subodontoblastic cell-dense zone. X 700.

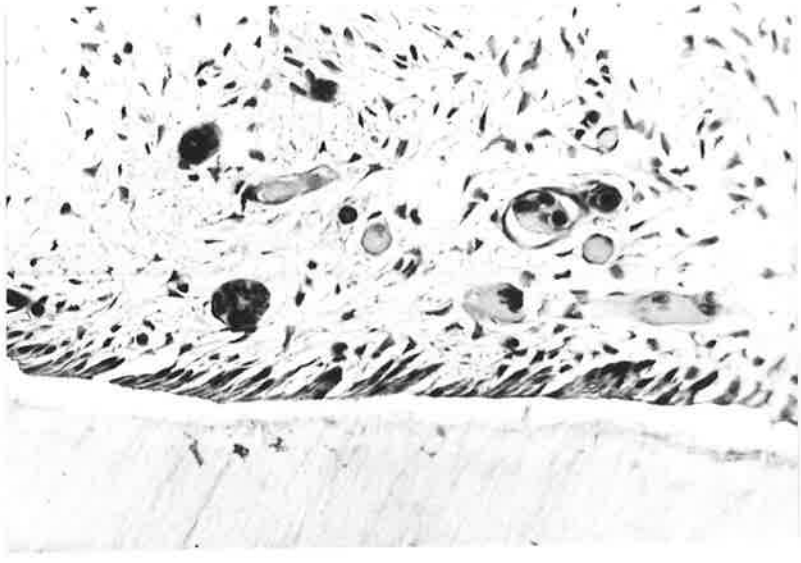


Fig. 36 Base of coronal pulp, same tooth as in Fig. 35, showing absence of subodontoblastic cell-dense zone. X 700.

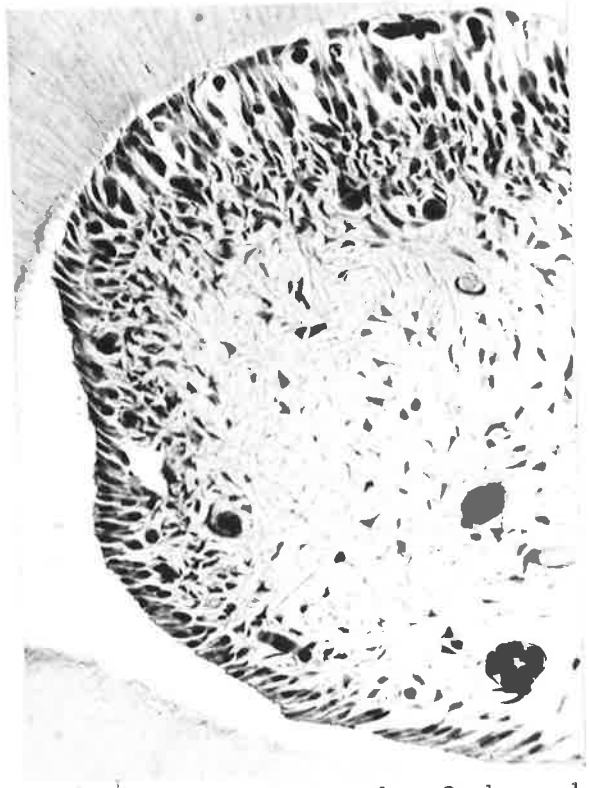


Fig. 37 Mesial aspect, coronal pulp, 2nd. molar showing selective distribution of subodontoblastic cell-dense zone. X 700.

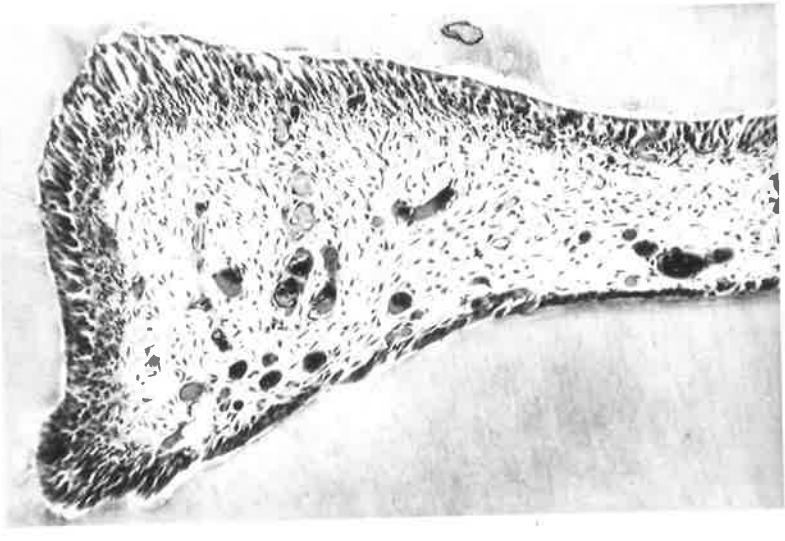


Fig. 38 Buccal aspect, coronal pulp, 1st. molar showing selective distribution of subodontoblastic cell-dense zone. X 430.

of the coronal pulp nor in the root canals (Figs. 27, 31, 36, 37, 38 and 39). The selective distribution of the subodontoblastic cell-dense layer is found not only in pulps of young animals but also in much older ones (Figs. 40 and 41). The development of this dense layer of cells is considered in detail in Chapter IV.

Mesenchymal Cells

The mesenchymal cells which make up the largest cellular component of the molar pulp are of a more differentiated form than those of the incisor, being true fibroblasts (Fig. 42). They are less numerous than in the incisor; consequently, there is a greater amount of intercellular material. In contrast to the situation existing in the incisor pulp and similar to that in the human pulp, their number decreases with age.

Connections between Pulp and Periodontal Membrane.

Lateral canals connecting the root canal pulp with the periodontal membrane are common, particularly in young teeth (Fig. 43). The pulp tissue in these communicating canals consists of fibroblast cells and intercellular material. At the apical end of the root canal the pulp may open into the periodontal

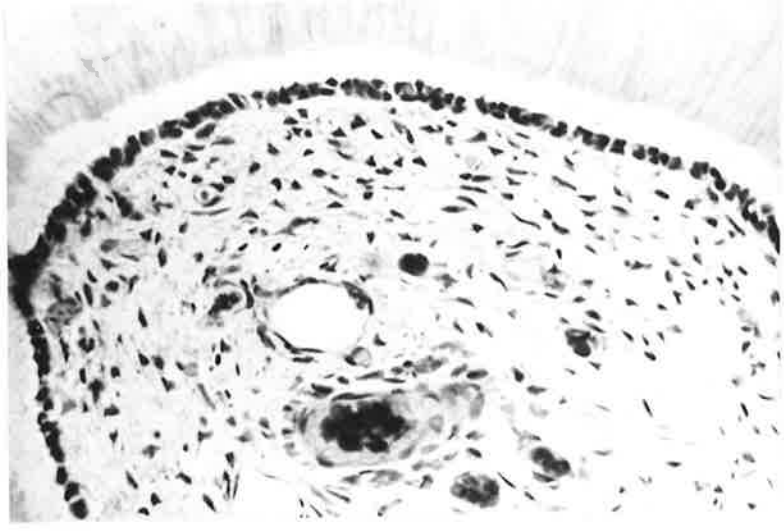


Fig. 39 Transverse section root canal pulp, 1st. molar showing even distribution of mesenchymal cells throughout pulp tissue. Absence of subodontoblastic cell-dense zone. X 800.

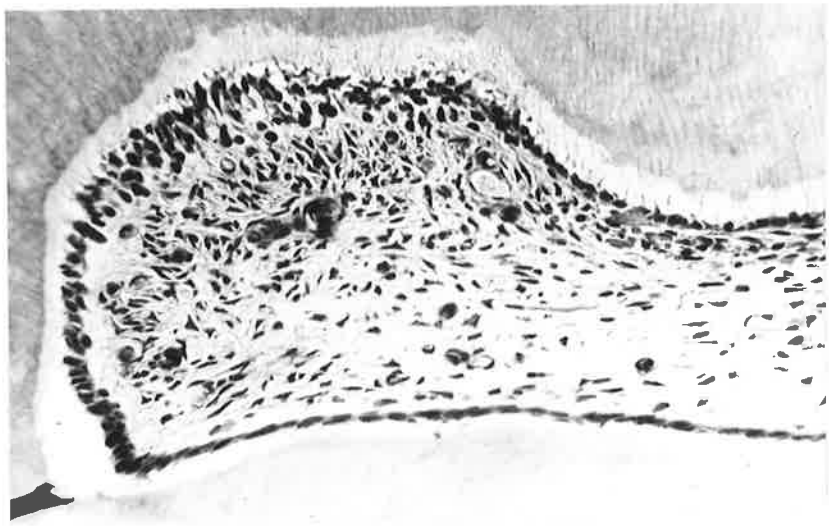


Fig. 40 Mesio-distal section, coronal pulp 1st. molar, 6-7 month old rat showing subodontoblastic cell-dense zone in roof and mesial aspect of pulp chamber, absence of such a zone in the base. X 800.

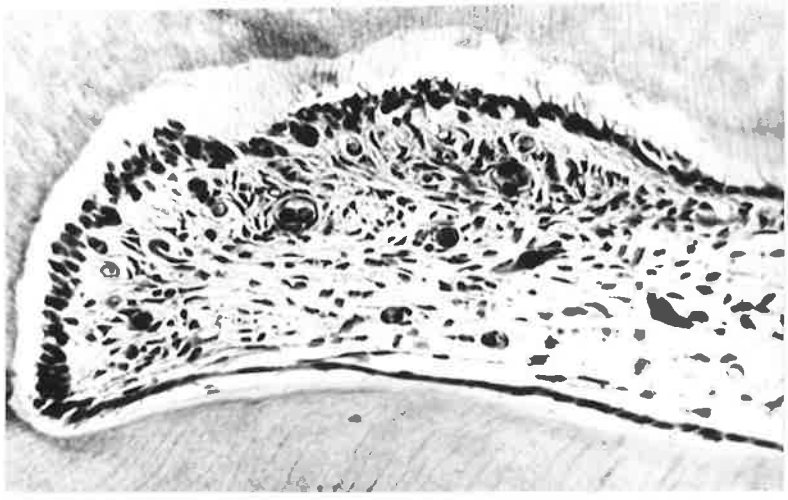


Fig. 41 Mesio-distal section, coronal pulp 2nd. molar, 6-7 month old rat showing selective distribution of subodontoblastic cell-dense zone. X 1100.

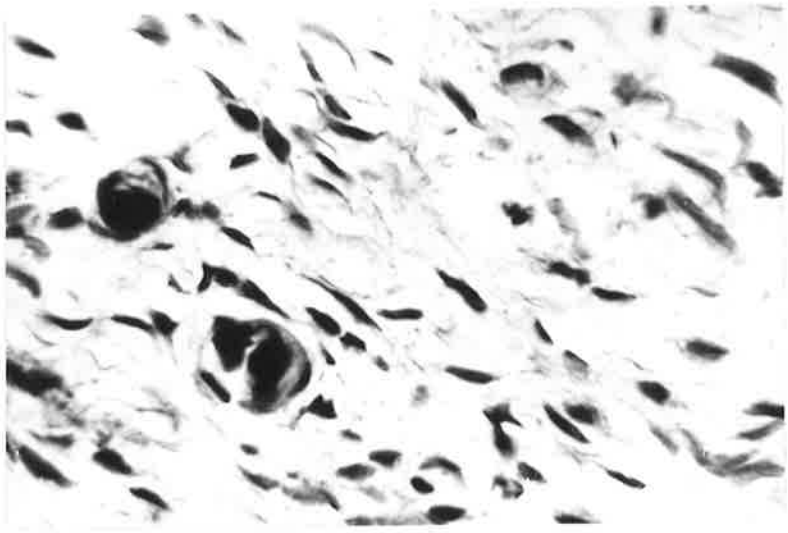


Fig. 42 Fibroblasts from coronal pulp of 6-7 month old rat. X 2000.

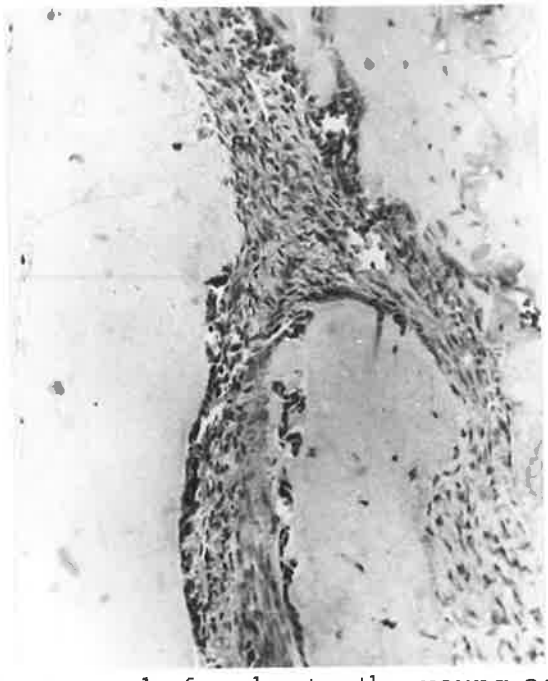


Fig. 43 Root canal of molar tooth, young adult rat, showing presence of lateral canal to periodontal membrane. X 800.

membrane directly at the apex or before the apex in a lateral location (Figs. 44 and 45).

DISCUSSION

It is evident that there is a considerable degree of similarity between the pulp of the rat molar and the human pulp, particularly the variation in odontoblast morphology which occurs in different anatomical locations, the decrease in the number of mesenchymal cells with increasing age and the existence of lateral connections between the pulp tissue and periodontal membrane. A common feature also is the great vascularity of the two tissues, particularly in the odontoblast cell layer. The dense network of capillaries between the odontoblasts is readily noted in sections of rat pulp and is in good agreement with the previously reported observations made from injection studies.

Some notable differences exist, however, between human and rat pulp tissue, the most significant being the absence of a distinct cell-free area beneath the odontoblasts in the coronal pulp. The cell-free zone in the human pulp has been shown by ORBAN, SCHOUR and others to contain nerve fibres. It is possible that it represents an important linkage in the sensory

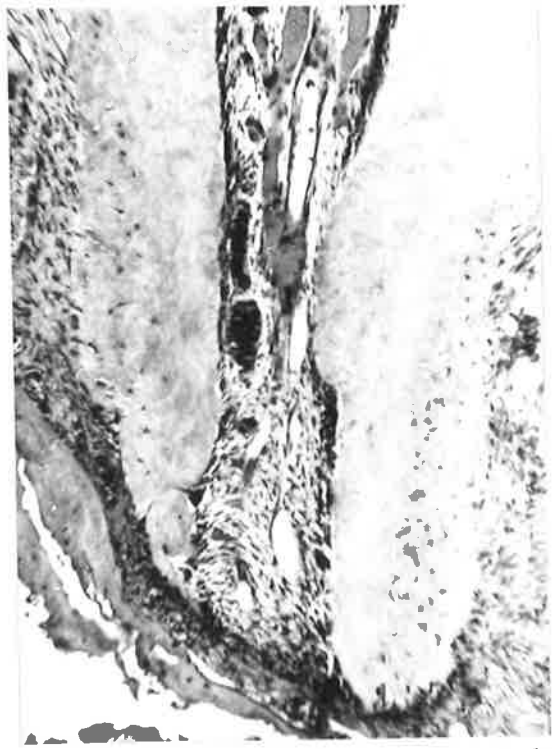


Fig. 44 Root canal of molar tooth, young adult rat, opening directly at apex into periodontal membrane. X 800.

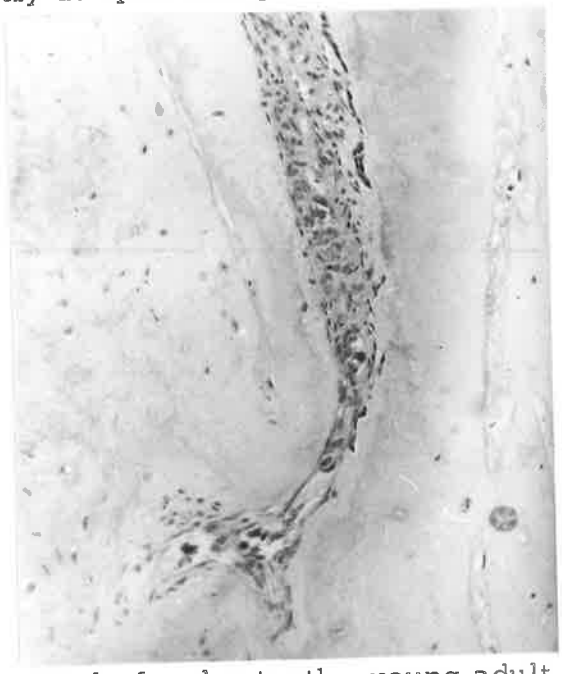


Fig. 45 Root canal of molar tooth, young adult rat, opening laterally before root apex. X 800.

mechanisms operating between dentine and pulp. The absence of a prominent cell-free zone in the rat pulp may be indicative of a less specialized sensory apparatus and may also be of evolutionary significance. Data is lacking, however, on the presence or absence of this zone in other species of animals.

It is readily apparent in sections of rat molar pulp that the distribution of a dense layer of mesenchymal cells beneath the odontoblasts follows a definite anatomical pattern, being confined to those regions of the coronal pulp lying directly beneath those dentinal tubules which radiate to the dentino-enamel junction. This cellular arrangement, which has not been commented upon in previous literature, is discussed more fully in Chapters IV and VI.

The fact that in the rat incisor pulp odontoblast and mesenchymal cells lie in close proximity while in the human pulp these two cell types are separated by a cell-free zone may partly explain why it is possible to detach the rat pulp from the dentine in a relatively intact form whereas this is not possible in the case of the human pulp. This cannot, however, be the complete explanation since in the root canal of the human pulp where the cell-free

zone is absent and mesenchymal cells lie adjacent to odontoblasts, the latter cells likewise do not come away with the pulp tissue but remain attached to the dentine. It is likely that the length of the odontoblastic process is an important factor in determining whether odontoblasts can be separated from dentine. In human teeth the process can be up to a few millimetres in length whereas in the rat incisor it is usually less than one millimetre.

It would appear that the procedure of dissecting the rat incisor pulp away from the dentine offers a satisfactory method for isolating and carrying out histochemical and cytological studies on odontoblast cells. This technique would overcome the problem encountered by ZERLOTTI (1946) who used human pulp tissue to study the histochemistry of its cells and intercellular material, but because of inability to remove odontoblasts with the pulp tissue, limited his observations to other pulpal cells only. The method could also aid in answering the problems outlined by NYLEN and SCOTT (1958). These authors stated: ".....further pertinent information could only be gained from electron microscopic studies of the odontoblasts during calcification of the matrix at the termination of primary

dentine formation, when their activity becomes relatively latent. Such investigations are planned, but there are a number of technical obstacles yet to be surmounted. The principal difficulty lies in the fact that it has not been possible to cut satisfactory sections of odontoblasts in the presence of calcified dentine. Since preliminary studies have shown that demineralization is damaging to cell structure, it will be necessary to perfect methods of microdissection which will permit isolation of the intact odontoblastic layer."

CHAPTER IV

CELLULAR ORGANIZATION IN THE SUBODONTOBLASTIC ZONE OF THE RAT INCISOR AND MOLAR PULP

INTRODUCTION

The presence of a prominent subodontoblastic mesenchymal cell-dense zone in certain regions of the coronal pulp of the fully developed rat molar was described in Chapter III. Reference was also made to a similar but less prominent cell-dense zone which is found beneath the odontoblasts in all regions of the pulp of the continually-erupting incisor tooth. This Chapter deals with the mode of development of the subodontoblastic cell-dense zones in the incisor and molar pulps and briefly discusses the significance of this characteristic cellular arrangement. A more detailed discussion of this feature of pulp histology is included in Chapter VI.

The Subodontoblastic Cell-Dense Zone in the Incisor Pulp

It was shown in the previous Chapter that a dense layer of mesenchymal cells can be found immediately beneath the odontoblasts in all regions of the pulp in the continually erupting incisor teeth of rats (Figs. 11, 12, 13, 14, 15, 20, 21 and 22). This layer of cells is usually more prominent on the labial than on the lingual aspect in the fully-functioning tooth. Developing, unerupted incisor teeth, however, do not exhibit significant differences in

cell-density between the subodontoblastic and the more internal regions of the pulp (Fig. 46). The subodontoblastic cell-dense zone in incisor pulp in regions away from the growing end becomes evident (Fig. 11) only after full development of the tooth has occurred, eruption has taken place and the process of attrition of the incisal edge has commenced following the establishment of functional occlusion of the incisor teeth.

The explanation for the presence of the cell-dense zone beneath the odontoblasts in the pulp of the fully functioning incisor tooth can be understood if one considers the manner in which the incisor pulp tissue is continually formed at the base of the tooth and moves toward the incisal region in a rhythmical manner, thereby replacing the tissue lost as a result of attrition.

At the base of the tooth in the regions of the labial and lingual dental papillae, large numbers of undifferentiated mesenchymal cells lie in close proximity to the epithelial tissues (Figs. 12 and 13). Some of these undifferentiated cells become incorporated into the odontoblastic layer. A large number of these, however, do not undergo differentiation, are not included in the odontoblastic layer, nor do they migrate into the central core of

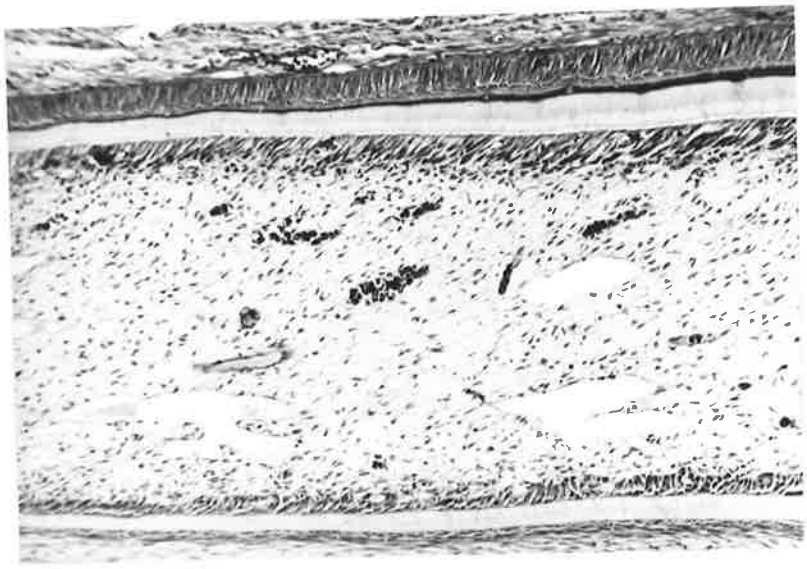


Fig. 46 Unerupted lower incisor, rat 8 days old showing absence of prominent subodontoblastic cell-dense zones in mid-region of pulp. (cf. Fig. 11)
X 280.

the pulp; instead they remain closely packed together in the subodontoblastic region. This layer of densely-packed mesenchymal cells remains beneath the odontoblasts and this cellular arrangement is perpetuated in the middle and incisal regions of the pulp by virtue of the forward movement of pulp tissue which occurs in response to loss of tissue at the incisal region and formation of new tissue at the base.

The Subodontoblastic Cell-Dense Zone in the Molar Pulp

The distribution of the dense layer of mesenchymal cells lying beneath the odontoblasts in fully developed molar pulps was described and illustrated in the previous Chapter. In contrast to the situation existing in the incisor pulp, the mesenchymal cell-dense zone in the molar pulp shows a selective distribution, being confined to those peripheral areas of the coronal pulp which are anatomically related to the external crown surface.

The aim of this part of the study was to determine, as far as possible, the period and mode of formation of the subodontoblastic cell-dense zone in the molar pulp. Examination of histological sections of developing teeth from animals a few days old

revealed that the mesenchymal cells of the pulp were evenly distributed throughout the whole tissue. No differences in cell density between the subodontoblastic and other regions of the pulp were evident (Figs. 47, 48, 49, 50 and 51).

At the time of exposure of the teeth to the oral fluids and before functional molar occlusion had occurred, a significant alteration in the distribution of mesenchymal pulp cells becomes apparent. The density of cells in the subodontoblastic zone in the roof and lateral aspects of the pulp chamber had increased (Figs. 52, 53, 54, 55, and 57). The increase became progressively greater with time (cf. Figs. 52 and 58) and a dense zone of mesenchymal cells lying immediately beneath the odontoblasts became quite prominent a few days after the teeth had been exposed to the oral environment (Figs. 58 and 59). These changes in cellular distribution were noted in all three molar teeth in upper and lower jaws. The formation of a subodontoblastic cell-dense zone in the roof and lateral aspects of the pulp chamber following eruption of the molar teeth was a constant finding.

Two mechanisms whereby the number of mesenchymal cells

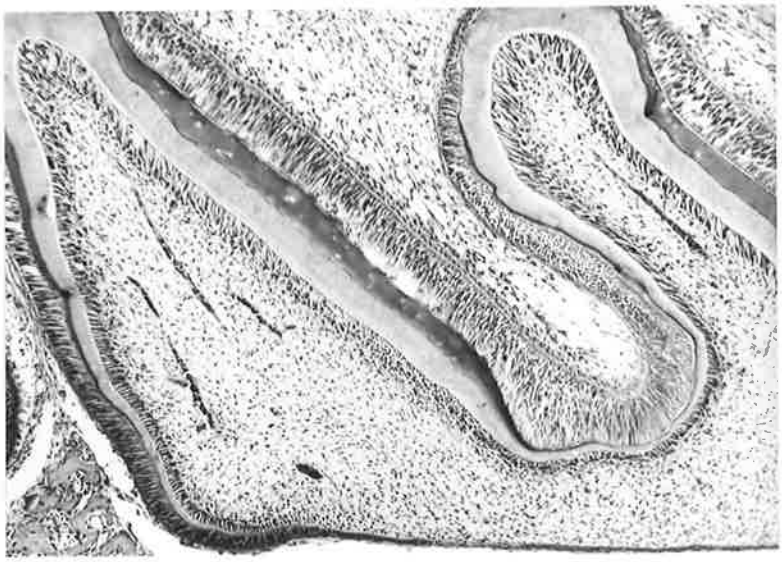


Fig. 47 Developing 1st. molar of rat 8 days old showing even distribution of mesenchymal pulp cells. X 220.

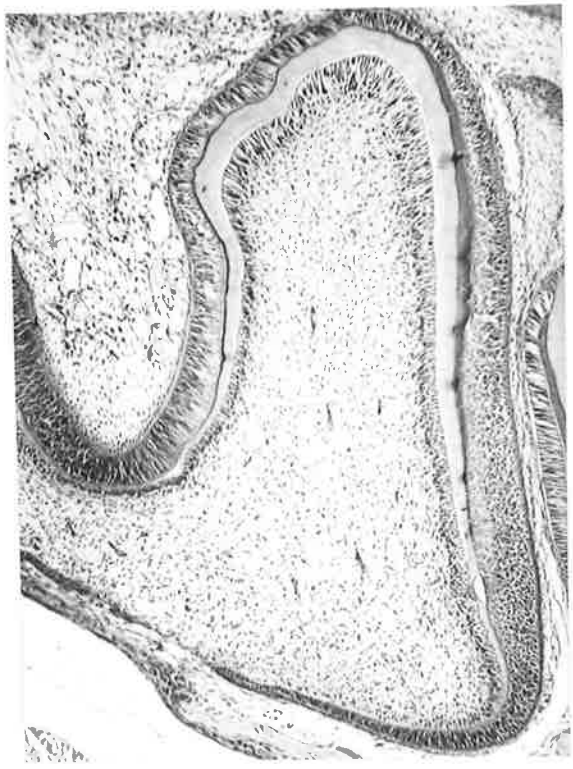


Fig. 48 Developing 2nd. molar of rat 8 days old showing even distribution of mesenchymal pulp cells. X 170.

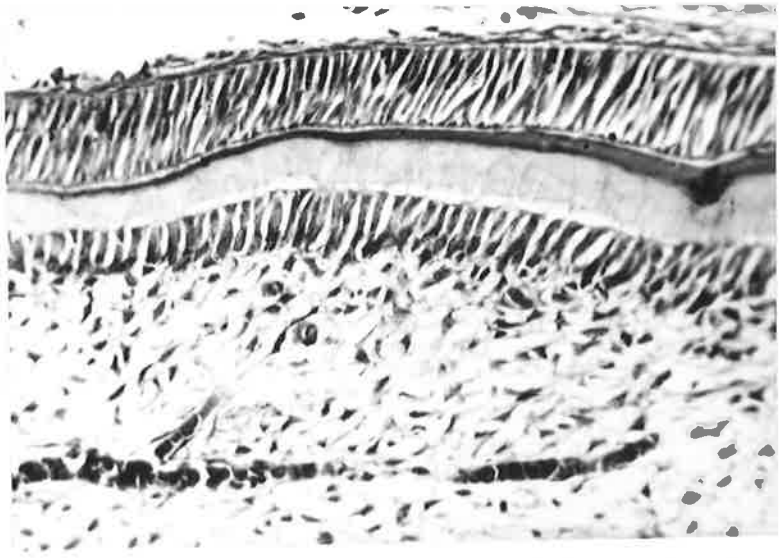


Fig. 49 Coronal pulp, 2nd. molar of rat 8 days old showing odontoblast cell layer and even distribution of mesenchymal cells. Absence of subodontoblastic cell-dense zone. X 1100.

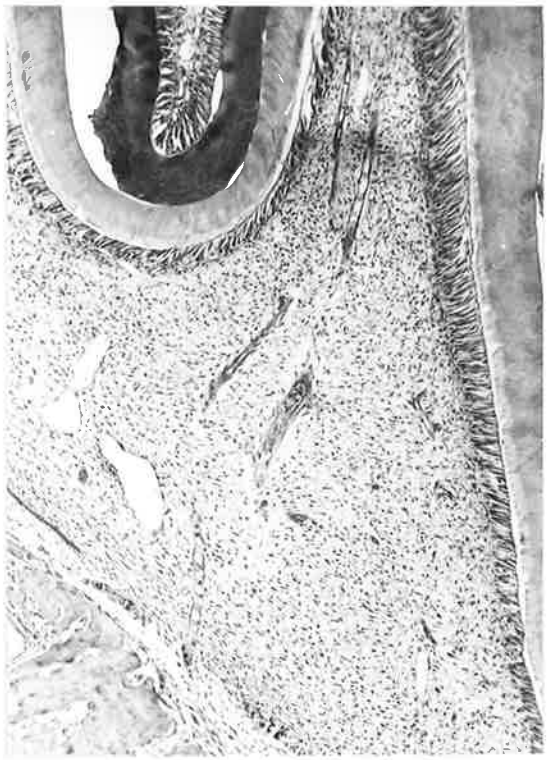


Fig. 50 Unerupted 1st. molar of rat 12 days old showing even distribution of mesenchymal cells. X 280.

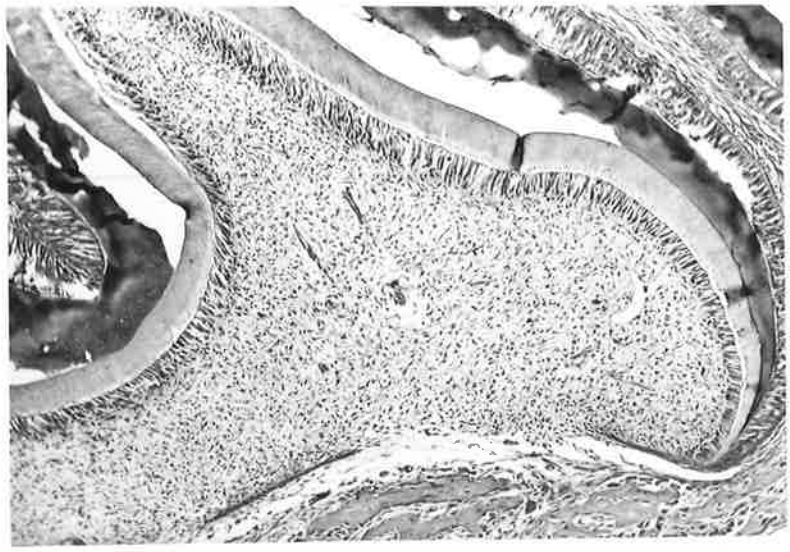


Fig. 51 Unerupted 2nd. molar of rat 12 days old showing even distribution of mesenchymal cells. X 280.

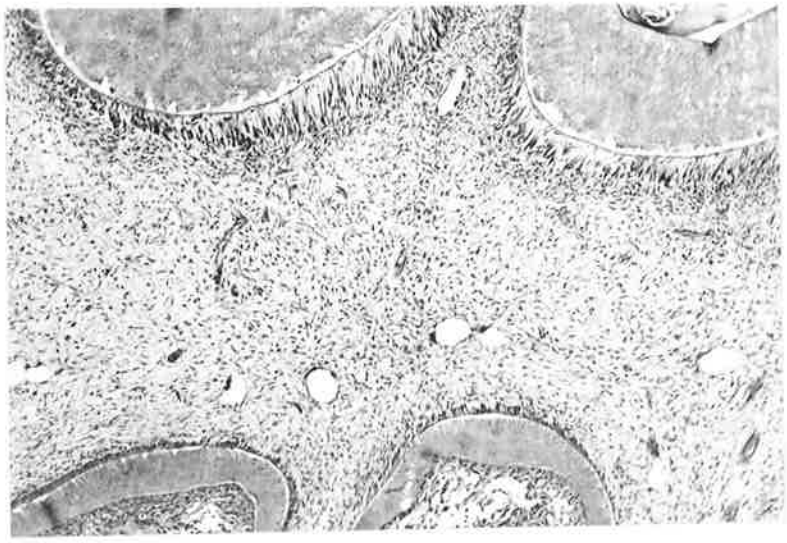


Fig. 52 Erupted 1st. molar of rat 20 days old showing early formation of subodontoblastic cell-dense zone in roof of pulp chamber. X 280.

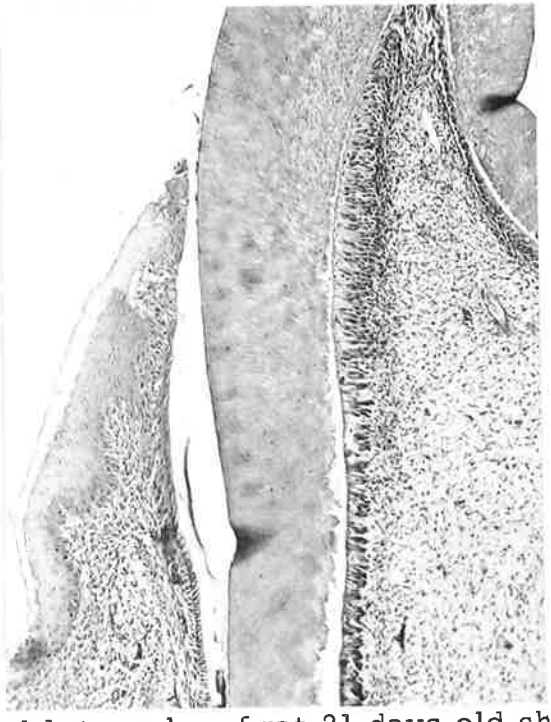


Fig. 53 Erupted 1st. molar of rat 21 days old showing early formation of subodontoblastic cell-dense zone in mesial aspect. X 280.

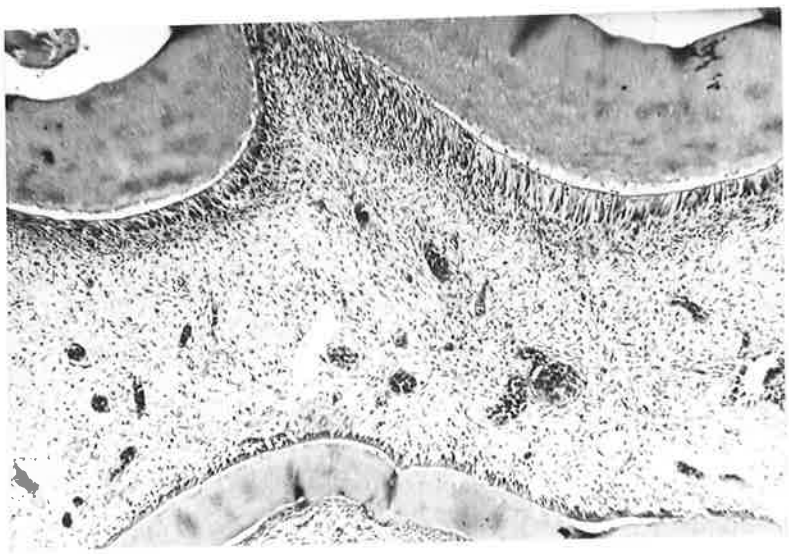


Fig. 54 Erupted 1st. molar of rat 22 days old showing prominent subodontoblastic cell-dense zone in roof of pulp chamber. X 280.

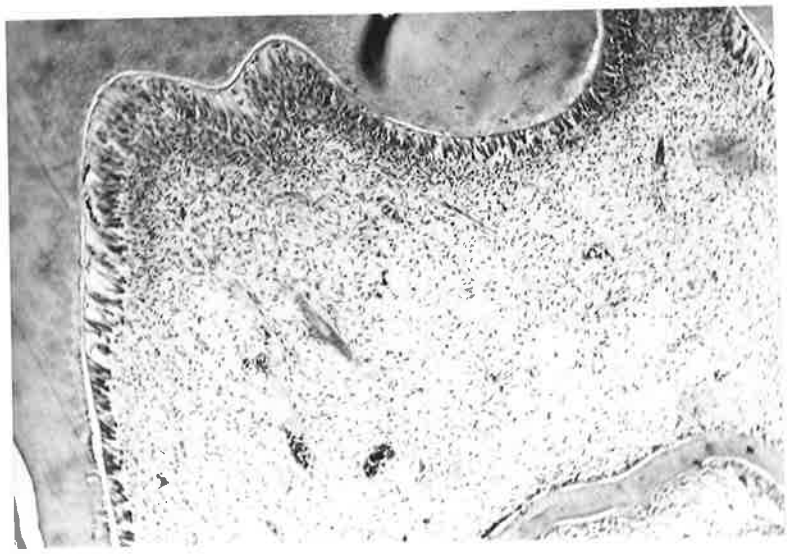


Fig. 55 Erupted 1st. molar at 24 days showing prominent subodontoblastic cell-dense zone in roof of pulp chamber. Density of cells in deeper regions of pulp appears to have decreased. X 280.

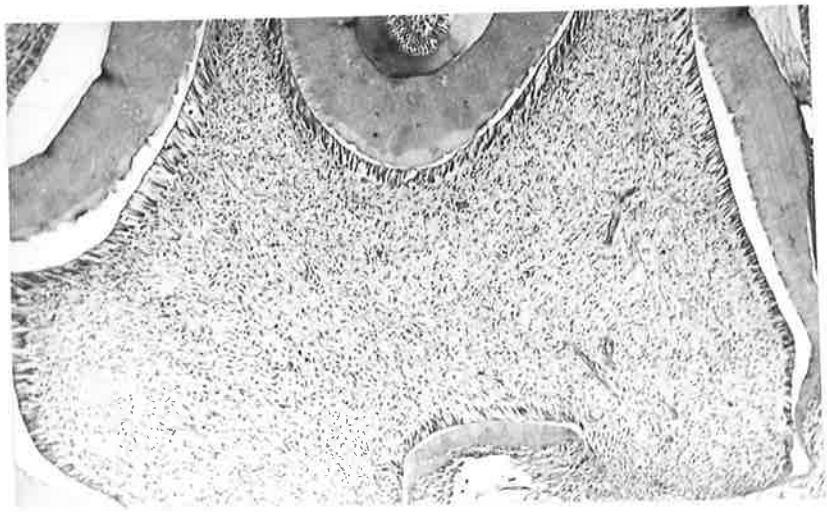


Fig. 56 Unerupted 2nd. molar of rat 21 days old showing even distribution of mesenchymal cells. Absence of subodontoblastic cell-dense zone. X 200.

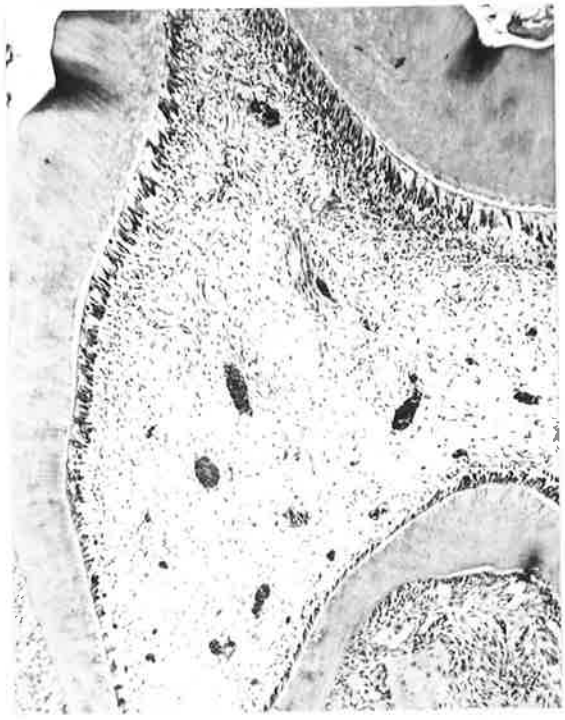


Fig. 57 Erupted 2nd. molar of rat 24 days old showing formation of subodontoblastic cell-dense zone in roof of pulp chamber and decrease in cell density in deeper areas. X 280.

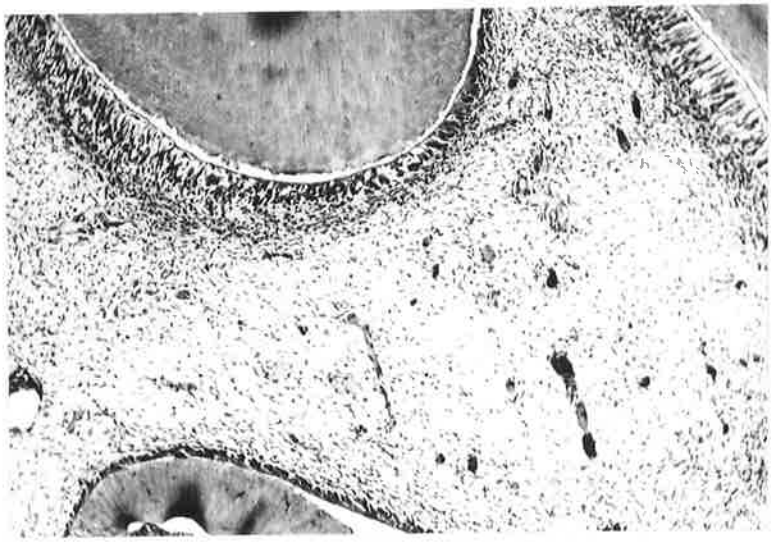


Fig. 58 1st. molar of rat about 5 days after eruption showing prominent mesenchymal cell-dense zone beneath odontoblasts in roof of pulp chamber. Density of mesenchymal cells elsewhere has decreased. X 280.

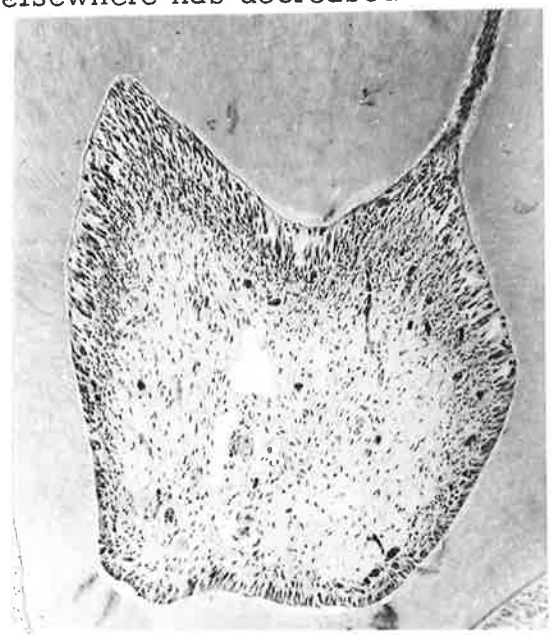


Fig. 59 3rd. molar of rat about 8 days after eruption showing prominent subodontoblastic cell-dense zone in roof, mesial and distal aspects of coronal pulp. X 280.

lying beneath the odontoblasts could increase were postulated, namely, increased mitotic activity and/or peripheral migration of cells from the more internal regions of the pulp. The latter explanation received support from the observation that coincident with the development of the subodontoblastic cell-dense zone, a decrease in cell-density occurred in the more central parts of the pulp (Figs. 55, 57, 58 and 59).

Further evidence in favour of the latter suggestion was provided by an experiment in which young animals of different ages received a single dose of colchicine throughout the period just prior to and following eruption of the teeth, in other words, throughout the whole period of development of the subodontoblastic cell-dense zone. Examination of molar pulp sections from these treated animals revealed that colchicine metaphases were an exceedingly rare occurrence within the subodontoblastic zone and in adjacent regions throughout this whole period, indicating that mitotic activity did not contribute to the formation of the cell-dense zone.

DISCUSSION

It is clear that the cell-dense zone found beneath the

odontoblasts in the pulp of the fully functioning incisor tooth is a manifestation of two processes which are characteristic of this tissue; namely, continual development at its growing end and forward movement of pulp tissue as a whole along the length of the pulp chamber toward the incisal region.

The molar teeth and their pulp tissues differ, however, from the incisors in that they are of limited growth and reach the end of their embryological development when root formation is complete. Consequently, differences in cellular arrangement which are found in various regions of the pulps of mature molar teeth can only be a reflection of processes occurring at these particular sites; they cannot represent a perpetuation of the situation existing in remote regions by virtue of mass movement of tissue, similar to that which occurs in the incisor pulp in response to functional loss of tooth substance. Another explanation is required, therefore, for the presence of the subodontoblastic cell-dense zone in the molar pulp.

The results of the present investigation have shown that the development of a dense layer of mesenchymal cells beneath the odontoblasts in the molar pulp begins at the time of eruption of

the molar teeth into the mouth. The cell-dense zone became evident firstly in the roof of the pulp chamber and subsequently became prominent also in the lateral aspects of the coronal pulp as complete eruption of the crown occurred.

Further investigation revealed that increased mitotic activity of mesenchymal cells is not the mechanism whereby the number of cells in the subodontoblastic zone increases. Rather, the formation of this zone results from a peripheral movement of cells which lie in the more deeper regions of the pulp. The observation that a decrease in cell density in the more central parts of the pulp occurred simultaneously with the development of the subodontoblastic cell-dense zone would be consistent with the suggested mechanism of formation.

The results obtained from this part of the study suggest that the formation of the subodontoblastic cell-dense zone in the rat molar pulp occurs in response to exposure of the tooth crown to the oral environment. Evidence is available from the radioisotope transfer studies of BARTELSTONE (1951) and SOGNNAES, SHAW and BOGOROCH (1955) to show that exchange of materials can occur in vivo between the pulp and salivary fluids across enamel

and dentine. Although evidence has not yet been brought forward, it is possible that such exchanges may be capable of influencing the structure of both the calcified tooth tissues and the dental pulp.

On the basis of results from the present investigation, it is postulated that exchanges between pulp and salivary fluids via enamel and dentine may mediate in some way the cellular organization within the subodontoblastic zone of the rat molar pulp. The selective redistribution of mesenchymal pulp cells during tooth eruption giving rise to a cell-dense zone in those regions of the coronal pulp where transfer of materials from salivary and pulpal environments can occur, would seem to favour this suggestion. The possible significance of the cell-dense zone is discussed in greater detail in Chapter VI.

CHAPTER V

CELLULAR PROLIFERATION IN THE DENTAL PULP OF THE ADULT RAT STUDIED WITH COLCHICINE AND TRITIATED-THYMIDINE-RADIOAUTOGRAPHY

INTRODUCTION

Several studies have been reported in which the general sites of proliferative activity in the rodent incisor pulp have been described. These investigations have already been reviewed (pp. 34-36). There is general agreement that the greatest amount of proliferation in the incisor pulp occurs in the basal region. In this location both undifferentiated (primitive mesenchymal) and partially differentiated (preodontoblast) cells undergo division. A few reports have also indicated that a low degree of mitotic division of mesenchymal cells occurs in regions remote from the growing end. However, no details are available on the precise sites of such mitotic activity. Information is lacking even further in the case of the molar pulp and only one brief report has appeared in the literature.

The object of this part of the investigation was to study accurately the precise sites of mitotic activity in the adult rat molar and incisor pulps. An important aim of the study was to examine the subodontoblastic cell-dense zones and adjacent regions for evidence of dividing cells. The cell-rich zone in the human pulp has been shown to sustain, by proliferation and differentiation,

the population of odontoblasts following injury to these cells (ZANDER, 1953; JAMES, SCHOUR and SPENCE, 1954; STANLEY, 1962). No data is available, however, to show whether division of cells in the cell-rich zone of human and rat pulps occurs in the absence of odontoblast damage resulting from external stimulation.

Results with Colchicine

Histological examination of tongue epithelium from all animals treated with colchicine revealed that the administration of the drug had been successful in causing metaphasic arrest of dividing cells. The actively proliferating basal epithelial layer showed numerous cells having the characteristic appearance of a colchicine metaphase, namely, the chromosomes clumped together in a dark mass in the centre of the cell and surrounded by a clear halo of cytoplasm (Figs. 60 and 61).

Incisor Pulp

The basal region of the pulp in continually-erupting upper and lower incisor teeth, the area in which active growth is occurring, showed as expected a considerable degree of mitotic activity (Fig. 62). Dividing cells were particularly numerous adjacent to

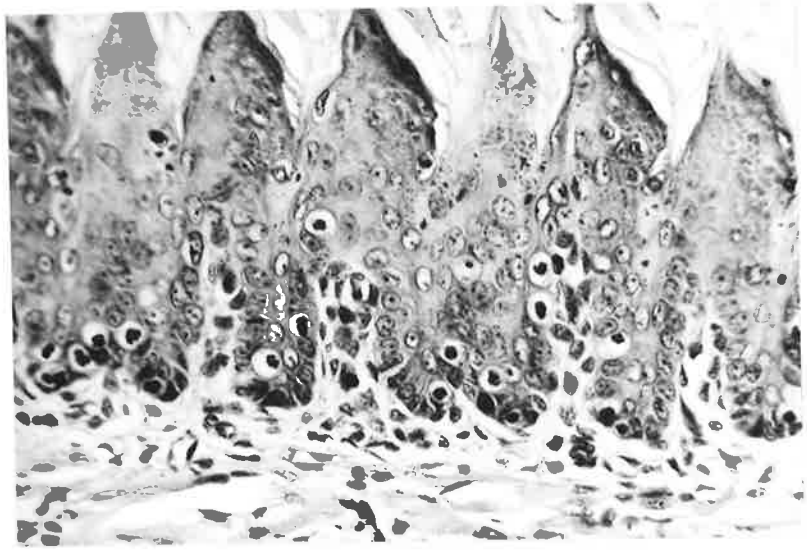


Fig. 60 Tongue epithelium of rat 20 days old given colchicine showing presence of colchicine mitoses in basal layer. X 800.

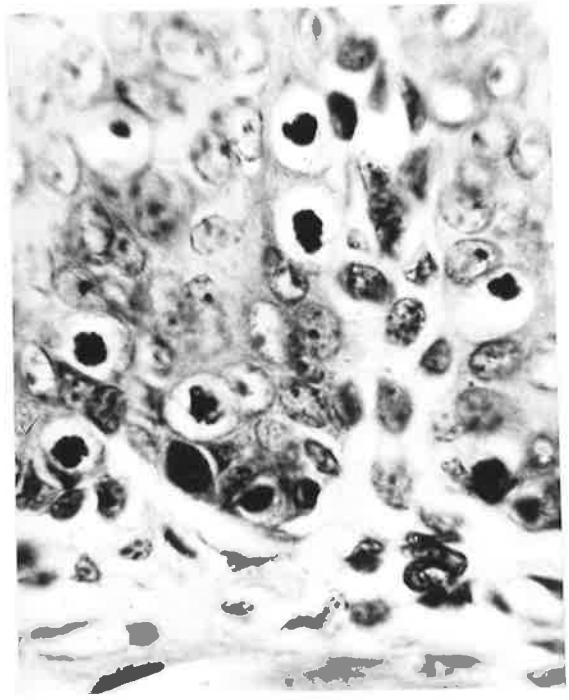


Fig. 61 Same tissue as shown in Fig. 60. Dividing cells which have been arrested in metaphase by the action of colchicine show chromosomes clumped together in dark mass surrounded by a clear halo of cytoplasm. X 2000.

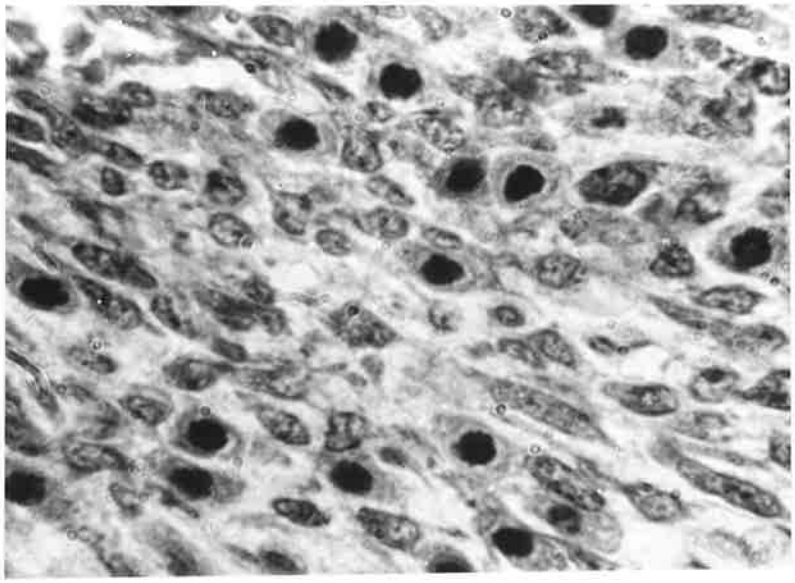


Fig. 62 Colchicine mitoses of undifferentiated mesenchymal cells in basal region, incisor pulp of young adult rat. X 2000.

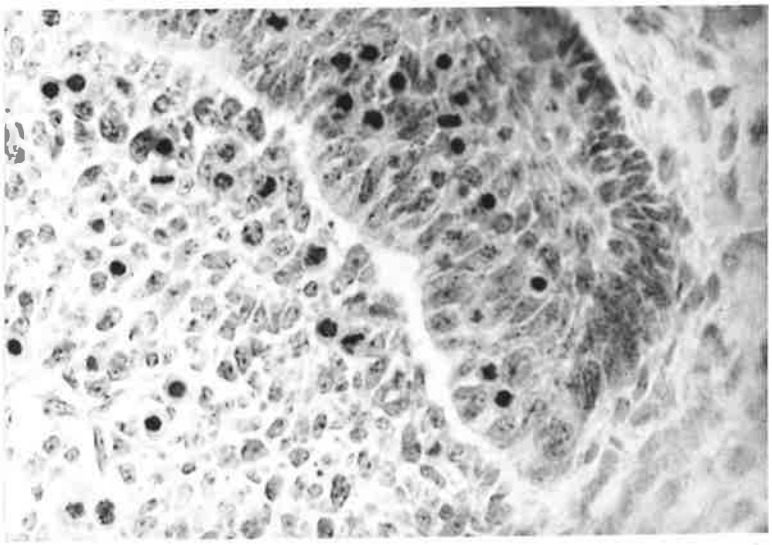


Fig. 63 Colchicine mitoses in preodontoblastic layer, basal region, incisor pulp of young adult rat. X 1100.

the labial (mainly) and lingual dental papillae. Mitoses were also observed within the newly-formed preodontoblast layer (Fig. 63). The mitotic indices (percentage number of dividing cells) obtained for the basal region of the pulp in the 4 groups of young adult animals (6 to 8 weeks of age) were as follows: Group 1 (12 noon - 6 p.m.), 2.75; Group 2 (6 p.m. - 12 midnight), 2.10; Group 3 (12 midnight - 6 a.m.), 2.46; Group 4 (6 a.m. - 12 noon), 3.26. In other words, a diurnal variation in mitotic activity occurred, with maximum activity being reached between early morning and noon and minimum between early evening and midnight.

The degree of mitotic activity decreased markedly with increasing distance from the basal end. Few dividing mesenchymal cells were found in about the middle third region of the pulp, and all of these were located in sites away from the sub-odontoblastic cell-dense zone. On rare occasions cells in the mature odontoblastic cell layer in about the middle region of the pulp showed the characteristic appearance of a cell in arrested metaphase (Fig. 64). Mitotic indices calculated for the middle third region of the pulp, however, were less than 0.1 in all groups of animals. No evidence of mitotic activity of odontoblast or mesenchymal cells was found in the region of the incisal pulp.

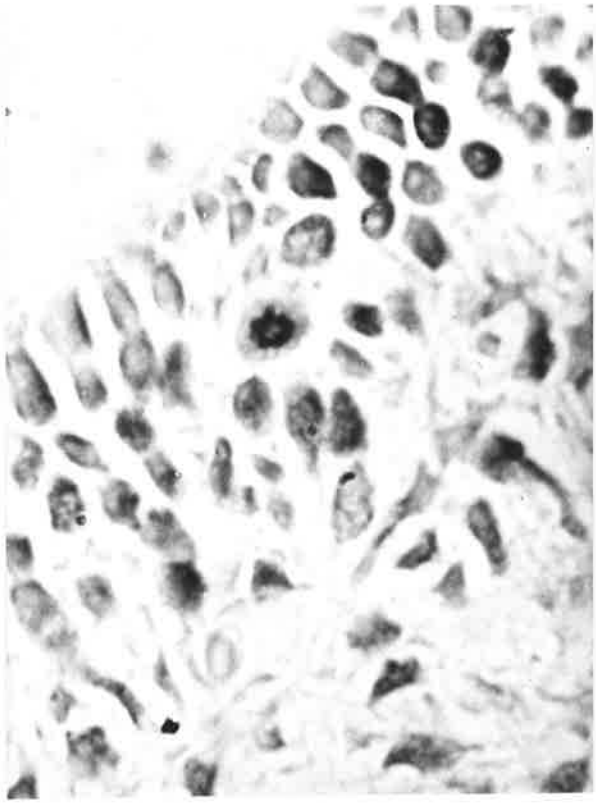


Fig. 64 Colchicine mitosis in mature odontoblastic cell layer, mid-region incisor pulp of young adult rat. X 2000.

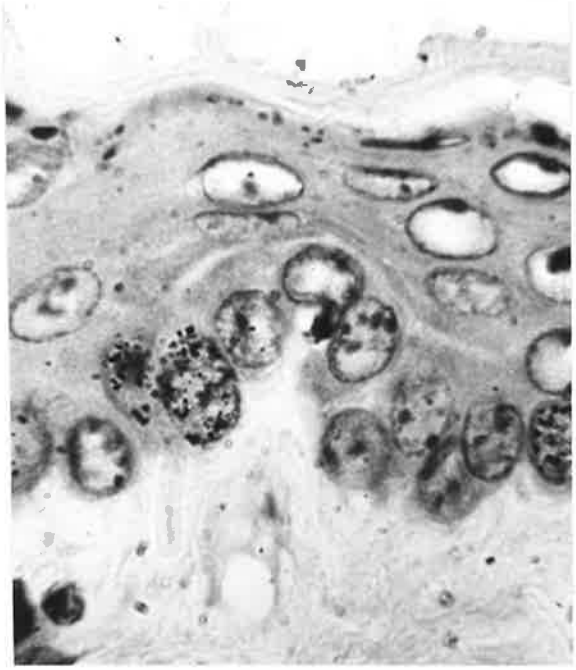
Results with older animals (6 to 7 months) again showed that the greatest amount of mitotic activity in the incisor pulp occurred in the basal region. The mitotic indices calculated for this region in the 4 groups of animals were as follows: Group 1, 2.52; Group 2, 2.44; Group 3, 2.41; Group 4, 2.47. These results show the same order of mitotic activity as in the corresponding region of the incisor pulp in young animals but with a less marked variation during different time periods over a 24-hour cycle. Middle and incisal regions of the pulp showed no mitosis of mesenchymal cells. Similarly, dividing cells in the mature odontoblastic layer were not observed.

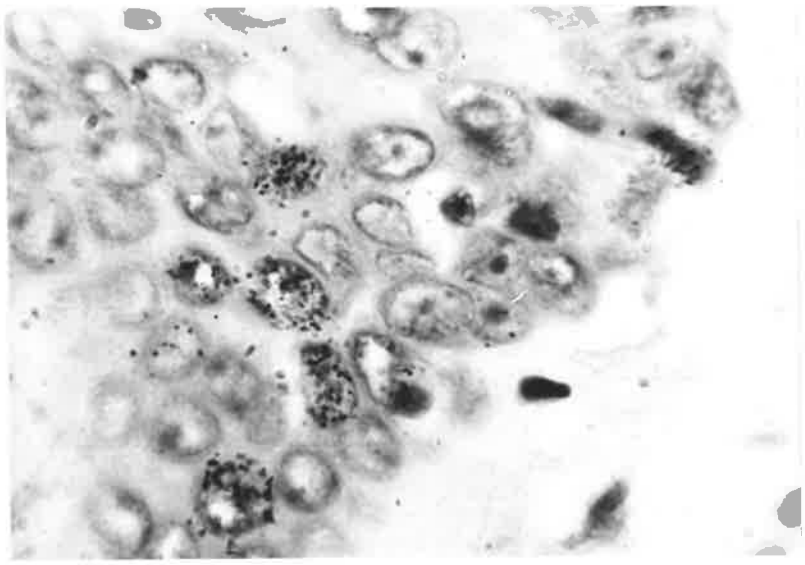
Molar Pulp

No mitotic activity of cells in the odontoblastic cell layer, subodontoblastic zone and remainder of the pulp tissue was noted in the molar pulps from young and old adult animals.

Results with H^3-Thymidine and Radioautography

Radioautographs of tongue epithelium from animals injected with H^3-thymidine showed good labelling of cells in the basal layer and a high degree of resolution (Figs. 65 and 66). Control radioautographs of tongue epithelium and teeth (i.e. those prepared





Figs. 65, 66 Radioautographs of tongue epithelia of young adult rats given H^3-thymidine showing presence of labelled cells in basal layers. X 2000.

from animals which had not received any isotope) showed no areas of significant blackening.

Incisor Pulp

Results of mitotic activity in the incisor pulp of young animals as revealed by the presence of labelled cells closely paralleled those obtained in experiment with colchicine. A high degree of mitosis was evident at the basal end of the pulp, particularly in areas of odontoblast differentiation (Fig. 67). Again a higher percentage of dividing cells was noted in the region of the labial dental papillae than on the corresponding lingual portions. Mitotic indices for the whole basal region in the 4 groups of animals were calculated as follows: Group 1 (12 noon - 6 p.m.), 2.45; Group 2 (6 p.m. - 12 midnight), 2.48; Group 3 (12 midnight - 6 a.m.), 2.55; Group 4 (6 a.m. - 12 noon), 2.58. The presence of labelled cells decreased rapidly away from the basal region and only a few labelled mesenchymal cells were noted in the middle regions of the pulp. These were located in the central region; labelled cells were not observed in the odontoblastic and subodontoblastic zones in regions remote from the growing basal end.

Radioautographs prepared from 6-month old animals which

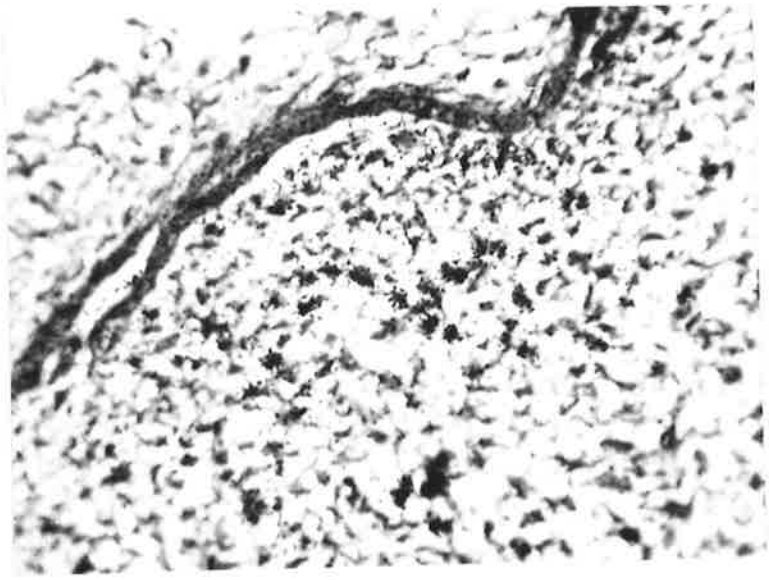


Fig. 67 Radioautograph incisor pulp of young adult rat showing large number of labelled cells in basal region. X 2000.

as foetuses and neonates, had received multiple doses of H^3-thymidine during the period of differentiation of cells responsible for development of the dentition, showed complete absence of labelled cells in all regions of the incisor pulp.

Molar Pulp

No significant degree of labelling was seen over cells in any region of the pulps from young adult animals. Radioautographs from 6 to 7 month old animals which had received multiple doses of H^3-thymidine during the early period of tooth formation revealed the presence of a few labelled mesenchymal cells in the coronal pulps of second and third molars (Fig. 68).

DISCUSSION

The results of the three short-term experiments with colchicine and H^3-thymidine-radioautography indicate that cells of molar pulp tissue from young and old animals are mitotically inactive. Further evidence of the mitotic stability of mesenchymal cells from molar pulps is shown by the presence of labelled cells in this tissue from 6-month-old animals which had received multiple doses of H^3-thymidine during the early developmental

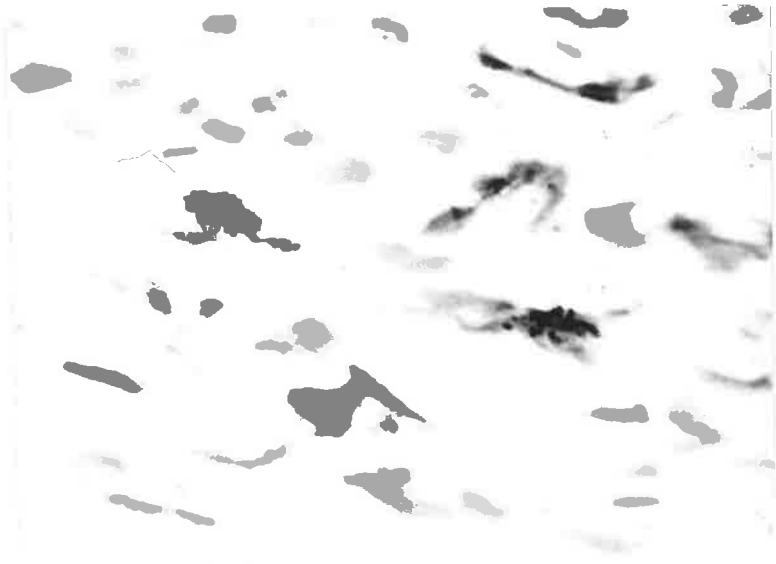


Fig. 68 Radioautograph molar pulp of 6 month old rat given H^3-thymidine during tooth development showing two labelled mesenchymal cells in coronal pulp. X 2000.

period of the teeth. Molar pulp tissue can, therefore, be classified as representing a static cell population.

Data obtained with both the colchicine and H^3-thymidine-radioautography techniques confirm the several previously reported observations that the greatest amount of cellular proliferation in the rodent incisor pulp occurs in the basal region and that in this location both undifferentiated and partially differentiated cells undergo division.

The results of the experiment on young adult animals using colchicine suggest that a diurnal variation in the degree of mitotic activity may occur, with maximum activity in the late morning and minimum in the evening. This variation, however, was not noted in the comparable experiment using H^3-thymidine nor was it evident in the case where older animals were studied with colchicine. Diurnal rhythms in mitotic rate can usually be attributed to variations in functional activity of a particular organ or tissue. One might expect that different patterns of feeding at different time periods by the animals used in the present investigation might have influenced the mitotic activity occurring at the base of the incisor teeth. However, the experimental animals were not observed

closely enough throughout the duration of the experiments to detect variations in feeding habits. Undoubtedly the experimental methods which involved one subcutaneous or two intraperitoneal injections per animal would have temporarily altered eating patterns to some extent.

The finding that a greater amount of proliferation occurs on the labial than on the lingual aspect in the basal region with consequent greater accumulation of cells in the labial subodontoblastic zone is of some considerable significance for it substantiates a point made earlier. In Chapter IV it was pointed out that the subodontoblastic cell-dense zone is usually more prominent on the labial than on the lingual aspect of the incisor pulp in regions remote from the growing end. In addition, the presence of the subodontoblastic cell-dense zone in areas of the pulp away from the growing region was explained by the continual forward movement of pulp tissue which occurs from basal to incisal regions in response to functional loss of tissue at the incisal tip. These three observations are, therefore, in good agreement with each other. Furthermore, the constant replacement of incisor pulp tissue is supported by the finding that labelled odontoblast or mesenchymal cells could not be found in radioautographs prepared from incisor

pulp tissue taken from 6-month-old animals which had received H^3-thymidine during foetal and neonatal life.

In agreement also with a number of previous reports is that the number of dividing cells decreases markedly with increasing distance from the basal end. Mitoses of mesenchymal cells in the middle and incisal regions of the pulp were a rare occurrence. Of further interest is that cells making up the subodontoblastic cell-dense zone exhibited no mitotic activity. An observation which has not been reported previously, however, is that a few cells in the mature odontoblastic layer may be capable of undergoing mitotic division. It has generally been believed that odontoblasts, being specialized cell types, are incapable of reproducing themselves by mitosis (ZANDER, 1953). Several authors have made reference to the presence of young cells in the odontoblastic layer (PAUL, 1899; MUMMERY, 1919; FISH, MANCH and MANCH, 1931; CABRINI and CABRINI, 1949; JAMES, SCHOUR and SPENCE, 1954). The cells in the mature odontoblastic layer of the incisor pulp which were observed to be undergoing mitosis in the present investigation may in fact represent immature odontoblast cells. Due to the loss of cytological characteristics during the mitotic cycle, however, it is not possible to make any firm deductions from these

observations as to the functional nature of these cells.

CHAPTER VI

GENERAL DISCUSSION

A feature of human pulp histology which has for many years been subject to constant description but very little discussion is the presence of structural layers in the coronal portion of pulps of fully-formed teeth. The cell-free and cell-rich zones lying beneath the odontoblast cells have been a common observation of several notable histologists, including ORBAN, SCHOUR and LANGELAND. These authors, however, have not made any suggestions as to the possible significance of this characteristic cellular arrangement apart from assigning a sensory function to the cell-free zone because of its content of nerve fibres. SCOTT and SYMONS (1958, 1961) have stated that the cell-free zone may be an artefact because of its absence in developing teeth and its varying appearance with different forms of histological processing. Comparative data from studies of other animal species have not been reported in the literature. Consequently, interpretation of the significance of observations made on human material has not been possible.

The preliminary investigation to the main text of this thesis has shown that histological processing of human pulp tissue in apposition with calcified dentine gives rise to an accurate histological appearance of the pulp. Distinct cell-free and cell-dense zones in the subodontoblastic region of the coronal pulp were

found to be present irrespective of whether the pulp tissue was processed while still maintaining its normal relationship to dentine or processed separately after separation from dentine. Under these different conditions of treatment, the cell-free and cell-dense areas were seen to be present in the coronal pulp but not in the root canal pulp. These layers cannot, therefore, represent an artefact. Furthermore, comparable results were obtained in the case of the rat incisor pulp. The appearance of the cell-dense zone beneath the odontoblasts was identical in decalcified sections and in sections of pulp tissue that had been dissected from the pulp chamber and processed without decalcification.

Results presented in this thesis of the comparative histology of the rat dental pulp from the neonatal to the adult periods of life have made it possible to put forward some suggestions regarding the significance of the cellular organization seen in the subodontoblastic zone of the pulp.

The adult rat molar pulp was found to exhibit a prominent subodontoblastic cell-dense zone in areas of the coronal pulp situated directly opposite those dentinal tubules which extended to the dentino-enamel junction. Cell-dense zones were not present

in the base of the coronal pulp nor in the root canals. Evidence of a cell-free zone in the coronal pulp was not found. The suggestion was made that the absence of a cell-free zone in the rat pulp may be indicative of a less specialized sensory mechanism than in the case of the human pulp and may also be of evolutionary significance.

Some preliminary observations from other comparative material lend support to these observations. Figs. 69, 70, 71, 72 and 73 in Appendix (v) of this thesis show the histological appearance of molar pulps from erupted deciduous rhesus monkey teeth. The subodontoblastic cell-dense zone appears quite prominent and is again limited to the roof and lateral aspects of the pulp chamber. The monkey pulp exhibits, therefore, the same selective distribution of the cell-dense zone as the rat pulp. A cell-free zone intervenes between the odontoblastic and cell-dense layers and appears to be a little less prominent than that of the human pulp.

A point of considerable significance is the fact that the selective distribution of mesenchymal pulp cells seen in sections of young and old adult rat molars was not evident in sections examined from neonatal animals. Pulp tissue from these latter animals showed a uniform distribution of mesenchymal cells with-

out any dense accumulations in the subodontoblastic zone. The formation of the cell-dense zone was observed to begin just prior to and during eruption of the molar teeth into the oral cavity. Peripheral migration of cells from the internal regions of the pulp tissue was shown to be the mechanism responsible for the development of the subodontoblastic cell-dense zone.

The formation of the cell-dense zone in areas of the pulp chamber which are linked to the external crown surface, its initiation during tooth eruption, and the active process by which it is formed, together with the evidence of the existence of a fluid interchange between the pulp and salivary environments, have formed the basis for the suggestion made earlier that the cellular organization in the subodontoblastic zone of the coronal pulp of the rat molar may be mediated by exposure of the tooth crown to the oral fluids. It is now necessary to discuss the subodontoblastic cell-dense zone in terms of its possible biological function in order to try and understand the significance of its formation.

The literature contains very few references regarding the significance of the cell-rich zone in the human pulp. These for

the most part relate to its role following odontoblast injury caused by pathological conditions and operative procedures. ZANDER (1953) stated that damage to odontoblasts by dental caries, abrasion, erosion and operative intervention resulted in a replacement of the injured cells by new odontoblasts. These new cells arose by differentiation of undifferentiated mesenchymal cells and young fibroblasts present in the cell-rich zone. ZANDER described the migration of these cells from the cell-rich zone, through the cell-free area and their incorporation into the odontoblastic layer.

JAMES, SCHOUR and SPENCE (1954) studied changes in cellular arrangement in the odontoblastic and subodontoblastic cell-layers following operative procedures. They also described in detail the morphological changes occurring in cells which migrated from the subodontoblastic cell-rich zone into the odontoblastic layer. Three hours following cavity preparation and insertion of gutta-percha filling material the number of odontoblasts beneath the operative zone appeared to be reduced, and the cell-free zone was invaded by cells from the cell-rich zone. These invading cells consisted of 'young fibroblasts' and a new type of cell which differed from the 'young fibroblast' in size and nuclear structure. The term

'preodontoblast' was used to denote these new cells. A few days following the operative procedure, 'preodontoblasts' entered the odontoblastic layer, were transformed subsequently into 'young odontoblasts' and about three weeks later had formed reparative dentine.

STANLEY (1962) observed that proliferation of cells in the cell-rich zone occurred following cavity preparation which was sufficiently traumatic to cause odontoblast damage. Mitotic figures were found as early as 24 hours after the operative procedure but were most prominent between three and twenty days. The cells which entered the odontoblastic layer gradually assumed the characteristic morphological features of odontoblasts. The cell rich zone was seen to undergo depletion in the number of its cells, having furnished the mesenchymal cells necessary for reconstructing the odontoblastic layer.

Studies on the proliferative aspects of healing of the rat pulp following damage brought about by operative intervention have been reported in brief by ZACH, TOPAL and COHEN (1965) and SVEEN and HAWES (1966). Both groups of workers used the H^3-thymidine-radioautography technique. ZACH et al. placed deep

cavity preparations in molar and incisor teeth and studied mitotic activity of pulpal cells up to 14 days later. Radioautographs examined at 14 days showed massive accumulations of reparative dentine in continuity with the cut tubules. 'Subodontoblastic cells' were observed to be heavily labelled. Odontoblast regeneration was said to occur between the eighth and fourteenth day following injury. SVEEN and HAWES found that mitotic activity of pulp cells was greatest between 4 and 6 days following odontoblast injury and concluded that 'fibroblasts..... contribute to the formation of new odontoblasts'.

It is apparent then, at least from human pulp studies, that cells within the subodontoblastic cell-dense zone play an important role in sustaining, by proliferation and differentiation, the population of odontoblasts should their number be reduced by an external injury. The results of the investigation reported in Chapter V of this thesis have shown that the subodontoblastic cell-dense zone in the rat molar and incisor pulp (in regions away from the growing base) is a mitotically-inactive cell layer under normal conditions. Probably the same situation applies to the cell-rich zone in the human pulp under normal conditions. The sub-odontoblastic cell-dense zone could be considered, therefore, to

represent a potential repair mechanism of the pulp against the possible danger of odontoblast damage resulting from external irritation. It is likely that the cells of the cell-dense zone are more differentiated than other pulpal mesenchymal cells and are capable of becoming incorporated into the odontoblastic layer and assuming the function of dentine-forming cells.

It must be pointed out, however, that because injured odontoblasts have been shown to be replaced by cells from the cell-dense zone, this does not mean that one of the prime functions of this cell layer is to replace odontoblasts which have been destroyed by external irritation. It is only reasonable to expect that replacement of injured odontoblasts would occur from cells lying closest to the injured area. Furthermore, from a teleological viewpoint, it is unacceptable to suggest a 'recognition' or an 'awareness' on the part of the pulp to the potential threat of odontoblast damage from dental caries, abrasion and erosion so that the number of cells in the subodontoblastic zone is increased as soon as contact of the tooth crown with the external environment is established.

The possibility exists, however, that odontoblast cells in

those regions of the pulp which show the presence of a subodontoblastic cell-dense zone may be subject to replacement during their normal life, that is, in the absence of external injury. It may be that odontoblasts which lie in areas where transfer of materials between external and internal environments can occur have a greater need to be replaced than odontoblasts in other sites. STEWART (1965) has claimed to have demonstrated a replacement of human odontoblasts by cells from the cell-rich zone during the tooth's life cycle. Replacement is said to occur through a simultaneous degeneration of the old cell body and joining together with the new cell so that the integrity of odontoblast cell body with the odontoblast process is maintained. Although little direct evidence for this mechanism occurring is available, nevertheless, the possibility must be considered. In this regard it is interesting to point out that a common observation in human coronal pulp tissue of teeth that have not been affected by pathological conditions is the presence of isolated cells in the cell-free zone (Figs. 1, 2 and 3). These cells could conceivably be in the process of replacing degenerated cells in the odontoblast layer. This, however, is pure speculation and requires more careful appraisal.

Further investigations are necessary before the full significance of the cellular organization in the subodontoblastic zone of the dental pulp is understood. It is planned to extend the present study and examine the comparative histology of the pulp in a wide variety of animal species with special reference to the subodontoblastic region. Ultrastructural, histochemical and tissue culture studies of odontoblast cells from different anatomic sites of the pulp, cells from the cell-dense zone and remainder of the pulp tissue would undoubtedly provide valuable information as to their morphological and functional significance. Additional experimentation is required to substantiate the concept of fluid communication between the salivary and pulpal environments via enamel and dentine. Successful heterotopic transplantation of developing tooth germs between members of the same strain of inbred rats would provide a method for checking the suggestion that the oral environment can influence the arrangement of cells in the subodontoblastic zone of the pulp.

The results of these and other future studies may bring to light many features of the biological processes to which the hard and soft tooth tissues are subject. Such knowledge may lead to a better understanding of not only the maintenance of normal health,

but aberrations of normality which manifest themselves as diseased states. It is to be hoped that this information will prove valuable in helping to formulate measures aimed at preventing the initiation of oral disease.

APPENDICES

APPENDIX (i)

Chronology of the development of the dentition of the albino rat.[1]

Developmental Process	Incisors	Molars[2] M - 1	M - 2	M - 3
Initiation	14th day in utero	13th day in utero	14-15th day in utero	20th day in utero
Histodifferentiation of Ameloblasts begins	18-19th day in utero	20th day in utero	21-22nd day in utero	8-10th day
Apposition of Dentine begins[3]	20-21st day in utero	20-21st day in utero	1-2nd day	13-14th day
Crown completed (Amelogenesis ends)	Growth continuous	11th day	13th day	21st day
Bifurcation of Roots begins	None present	14-16th day	16-18th day	24th day
First appearance in oral cavity	About 10th day	19th day	22nd day	35th day
Functional occlusion	16th day	25th day	28th day	40th day

1. Data abstracted from: ADDISON and APPLETON (1915); GLASSTONE (1936); MELLANBY (1939); HOFFMAN and SCHOUR (1940).
2. Upper teeth are chronologically about 24 hours behind the lower.
3. Amelogenesis begins 12-24 hours later.

This information on the albino rat has been taken from FARRIS and GRIFFITH (1942). Eruption times of teeth of Hooded-Wistar rats used in the present investigation were found to conform closely with the above-listed times.

APPENDIX (iii)

Histological Techniques

Fixatives

(a) Bouin's Fluid

Saturated aqueous solution of picric acid	75 ml
Formalin (40 per cent formaldehyde)	25 ml
Glacial acetic acid (added immediately before use)	5 ml

(b) Neutral Formol-Saline

Formalin (40 per cent formaldehyde)	10 ml
Sodium chloride	0.9 g.
Distilled water	to 100 ml.

Solution buffered to pH 7 by addition of 3.5 g of sodium dihydrogen phosphate (anhydrous) (NaH_2PO_4) and 6.5 g. of disodium hydrogen phosphate (anhydrous) (Na_2HPO_4) per litre of the diluted fixing solution.

(c) Zenker's fluid

Mercuric chloride	5 g.
Potassium dichromate	2.5 g.
Sodium sulphate	1 g.
Distilled water	to 100 ml
Glacial acetic acid (added immediately before use)	5 ml

Decalcifying Fluid

Formic Acid	25 ml
Formalin	5 ml
Distilled water	70 ml

APPENDIX (iii) (continued)

Stains

(a) Ehrlich's Haematoxylin

Haematoxylin	6 g.
Absolute alcohol	300 ml
Distilled water	300 ml
Glycerol	300 ml
Glacial acetic acid	30 ml
Potassium alum	(added until deposit of crystals forms on bottom of container)

(b) Eosin

1 per cent aqueous solution of water-soluble eosin.

APPENDIX (iv)

Papers presented in support of this thesis:

The Mitotic Activity of Dental Pulp Cells.

T. Gotjamanos, The University of Adelaide.

Paper read before the International Association for Dental Research, Australian Section, Fifth Annual Meeting, Brisbane, 24th., 25th., 26th., August, 1965.

Cellular Organization in the Subodontoblastic Zone of the Dental Pulp

T. Gotjamanos, The University of Adelaide.

Paper read before the International Association for Dental Research, Australian Section, Sixth Annual Meeting, Sydney, 23rd., 24th., 25th., August, 1966.

APPENDIX (v)

Extension of the study: Comparative histology of the Rhesus monkey pulp.

Preliminary investigation has revealed that the cellular arrangement in the pulp of deciduous rhesus monkey molars has some features in common with human and rat molar pulps. Fig. 69 shows the odontoblastic layer, cell-free zone and a very prominent subodontoblastic cell-dense zone in the coronal pulp of a first molar. Figs. 70, 71, 72 and 73 show that the distribution of the cell-dense zone follows the same selective anatomical pattern as in the case of the rat molar, namely in the roof and lateral aspects of the coronal pulp.

Figs. 69, 70, 71, 72 and 73 produced from the collection of Dr. J. A. Cran. Photomicrographs reproduced by kind permission.

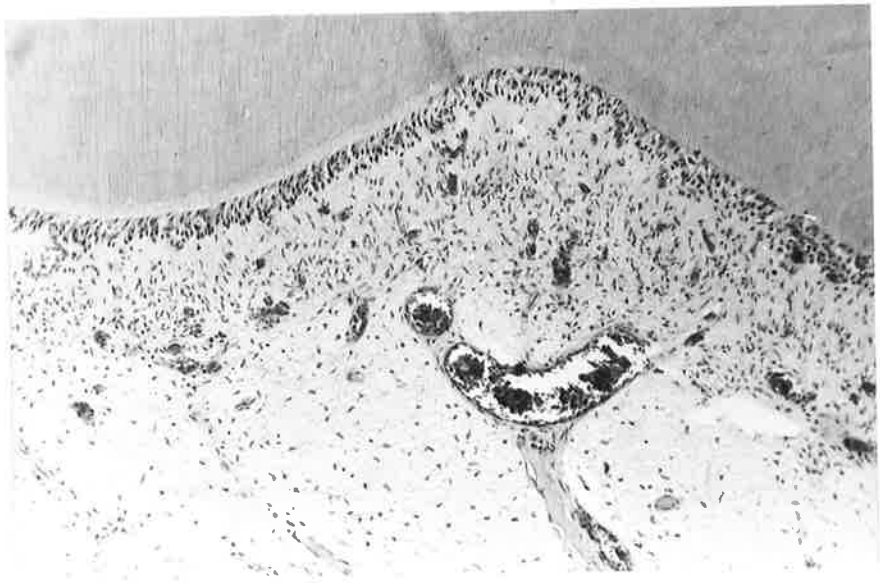


Fig. 69 Coronal pulp 1st. deciduous molar of rhesus monkey showing subodontoblastic cell-free and cell-dense zones X 430.

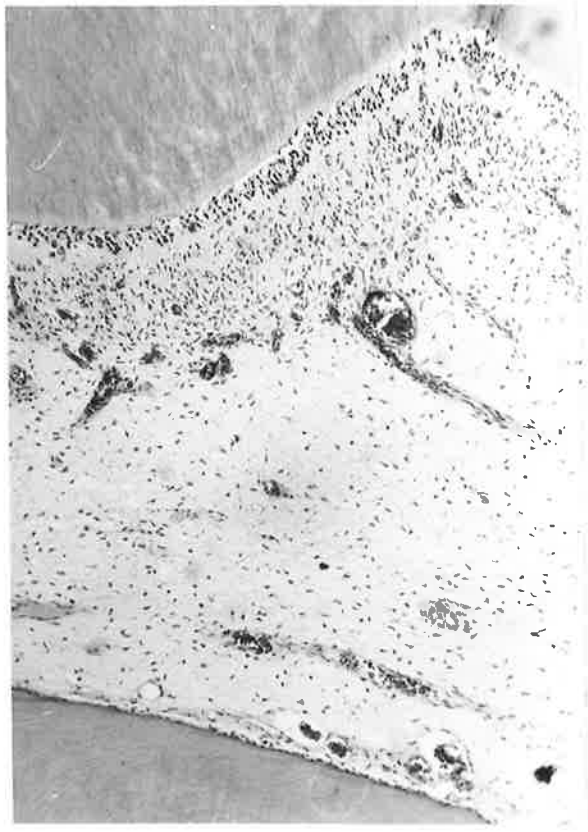


Fig. 70 Coronal pulp 1st. deciduous molar of rhesus monkey showing subodontoblastic cell-free and cell-dense zones in roof of pulp, and absence of such zones in base. X 300.

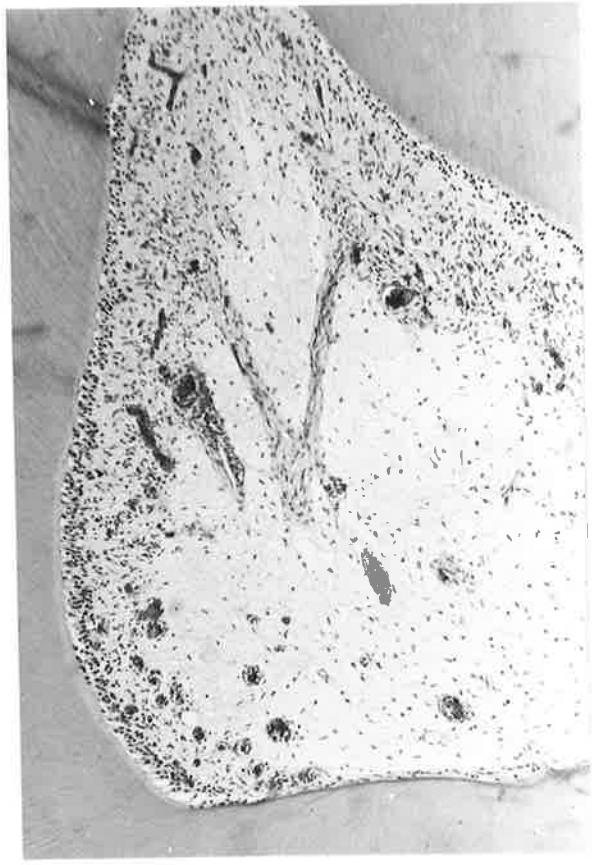


Fig. 71 Mesial aspect of coronal pulp of 1st. deciduous molar, rhesus monkey showing selective distribution of subodontoblastic cell-dense zone. X 300.

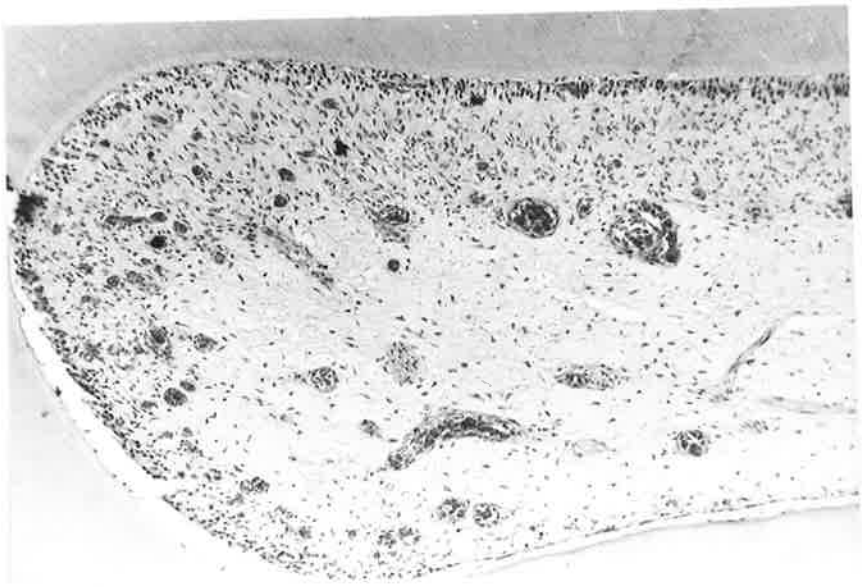


Fig. 72 Mesial aspect, coronal pulp, 2nd. deciduous molar, rhesus monkey showing selective distribution of subodontoblastic cell-dense zone. X 300.

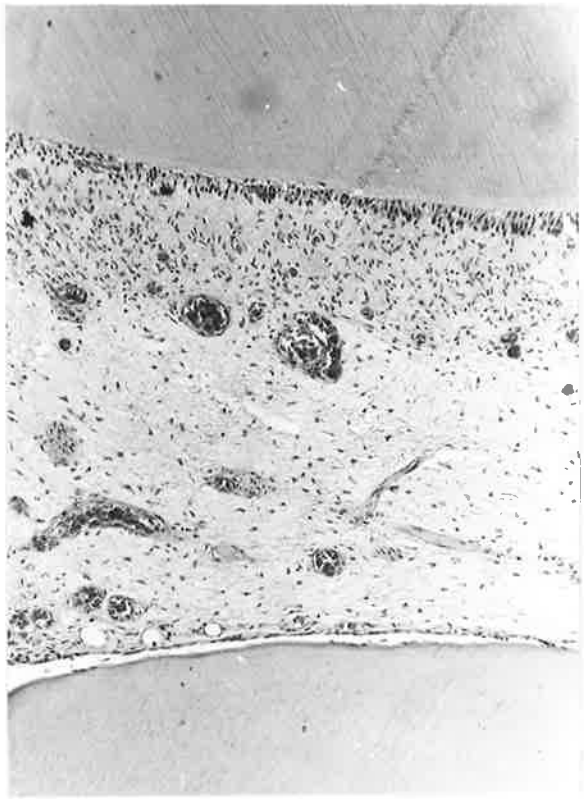


Fig. 73 Coronal pulp, 2nd. deciduous molar showing distribution of cell-dense zone. X 300.

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