T H E U N I V E R S I T Y O F M I C H I G A N

SCHOOL OF DENTISTRY

Progress Report

NERVE CONDUCTION IN HUMAN TEETH

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A. SUMMARY PAGE

1. Research Grant Number and Short Title:

USPHS-DE-01604 Nerve Conduction in Human Teeth

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4. Period Covered by Report:

April 1, 1964 to July 1, 1966

5. Date of Preparation of this Report:

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6. Summary Statement:

The work in the past period has been concentrated in five areas. (1) The study of nerve distribution in the pulps of deciduous teeth in stages of root formation, root completion and resorption was completed during this period. It was found that as the roots form, the nerves organize into central trunks and peripheral plexus. As the pulp matures a distinct cell-rich zone appears. As resorption begins the fine nerves disappear early in the process and only the major trunks and branches remain. With progressive root resorption, the large neural components undergo fragmentation, thickening, varocosities and finally disappear. This work was submitted for publication to the Anatomical Record. (2) Studies on the concentration and localization of acetylcholinesterase in the formed and resorbing human deciduous teeth were also completed this past period. The concentration of cholinesterase was greatest in the non-resorbing teeth and decreased in amount as the teeth underwent resorption. (3) Another project completed during this period was the analysis of cholines-

terase in 200 freshly extracted teeth. The teeth, ranging in age from 12 to 65 years, were freshly extracted, homogenized, and gel electrophoretic techniques carried out using AChE, BuThCh and naphtholic substrates and D.F.P. and eserine sulfate inhibitors. Evidence of the presence of cholinesterase in the pulps of various ages was determined by these means.

(4) Experimental studies were also carried out during this period. The inferior alveolar and cervical sympathetic nerves of the mandibular teeth of 24 New Zealand white rabbits were unilaterally resected. Cholinesterase determinations by gel electrophoresis and histochemistry as well as staining of neural elements was carried out. This work clearly revealed the diminution of neural elements and of acetylcholinesterase. This work is in progress and is currently being correlated with ultrastructural, cytochemical and cytologic findings.

(5) Ultrastructural studies were begun during this past period and the characteristics of the neural elements of the pulp as well as the dentin are being examined. The stages of early dentinogenesis have been studied in the hope of learning more about the characteristics of the neural conduction systems development and early function. Future plans include continued studies of all the components in dentin that may function in transmission of impulses thru that tissue. These studies will be carried out in the developing as well as the aging system and after nerve resection or trauma.

B. DETAILED REPORT

1. DESCRIPTION OF RESEARCH

Acetylcholinesterase in Human Primary Teeth

The mechanism of pain conduction in human teeth is not yet well understood. Many investigations of this subject have utilized the techniques of silver staining of the neural tissues.[1-4] These stains are not specific for neural elements, however, and this has presented problems of interpretation when they are visualized with other argyrophilic structures and in association with calcified tissues. Investigations have shown that chemical substances, known to be associated with the transmission of neural impulses, are present in human permanent teeth, Avery and Rapp.[5,6] These studies have been based on evidence that acetylcholine (ACh) is associated with the conduction of an impulse. Elliott[7] in 1904 hypothesized that a stimulated nerve released a chemical substance and Loewi[8] in 1921 demonstrated the presence of such a substance, which he termed Vagustoff. This chemical was isolated and chemically characterized as acetylcholine in 1929 by Dale and Dudley.[9] The concept of acetylcholine as a substance functioning in neural impulse transmission along a nerve and across a synapse is now widely accepted. Although the exact mechanism is not yet understood, it is believed the action of ACh is in causing an alteration of the permeability of the neural membrane to sodium ions which in turn generate a bioelectric potential. Acetylcholine is probably synthesized in the mitochondria located in the cell body, axon and dendrite (Hebb and Smallman, 1956)[10] and is possibly stored in an inert form within these tissues (Mann, Tennenbaum and Quastel).[11] An enzyme, acetylcholinesterase (AChE), is present in high concentration in neural tissue, hydrolysis acetylcholine, and causes a termination of the impulse transmission. The presence of choline, acetylase, acetylcholine and acetylcholinesterase in nerve tissue as well as the rapidity in which the acetylcholinesterase inactivates acetylcholine provides evidence of the chemical reaction working in conjunction with the electrical transmission.

Histochemical identification of AChE indicates the presence of ACh and thus the pathway of neural conduction. Cholinesterase (ChE) were initially demonstrated by Plattner,[12] and currently many types of these substances are known to exist. AChE, the specific ChE, is found in the brain, at synapses, motor end-plates and along the entire neural conducting membrane.

Zajicek, et al.,[13] have observed AChE in red blood cells and Stedman, et al.,[14] and Koelle[15] have found non-specific ChE in the serum of the blood. The cholinesterases are differentiated by their selective actions on various substrates. Specific ChE hydrolyzes acetylthiocholine (AThCh) but not butyrylthiocholine (BUThCh). Non-specific ChE will hydrolyze both the butyrylthiocholine and the acetylcholine substrates. Further differentiation

can be achieved by the use of inhibitors such as di-isopropyl fluorophosphate (DFP) or eserine sulfate which inactivates the enzymes totally or partially.

Clinicians are aware that primary teeth are less sensitive to operative procedures than permanent teeth. Rapp, Avery and Strachan,[16] in a study of nerves of primary teeth, revealed distribution of neural elements to be similar to that of permanent teeth but considerably less in number. A decrease in the number of small nerve fibers of primary teeth during the process of root resorption was also observed.

The purpose of the present study is to investigate the presence and location of AChE in the pulps of human primary teeth with complete roots and compare this to the pulps of teeth undergoing root resorption. Finally this study will attempt to relate the levels of clinical observation of these chemical substances to the levels of sensitivity of exfoliating teeth. A better understanding of the mechanism of pain in these teeth should result.

MATERIALS AND METHODS

The histochemical method, described by Koelle and Friedenwald[17] and subsequently modified by Pearse,[18] Churchill, et al.,[19] and Avery and Rapp[6] was employed to demonstrate specific and non-specific ChE in human primary teeth (Table 1).

TABLE 1

TECHNIQUE USED TO DIFFERENTIATE SITES OF ACTIVITY OF SPECIFIC AND NON-SPECIFIC CHOLINESTERASE IN HUMAN PRIMARY TEETH

TISSUES	INHIBITOR	SUBSTRATE	ENZYME LOCALIZED
Group 1	DFP	AThCh	Specific ChE
Group 2		BuThCh	Non-specific ChE
Group 3			Non (Control)
Group 4	DFP	BuThCh	Non (Control

Thirty-five freshly extracted human primary cuspids and molars, both resorbing and non-resorbing, were collected and frozen in saline until ready for use. Following sectioning at 100 to 200 microns on a calcified tissue cutting machine, the tissues were placed in the following solutions of sodium sulfate alone or containing an inhibitor until incubation began. The tissue sections were divided into four experimental groups. Group 1 was pretreated with 10^{-6}M DFP for 30 minutes at 37°C to inhibit completely the non-specific ChE while reducing the

activity of the specific ChE by 40 per cent. Incubation followed in a substrate of AThCh and copper ions for 20 hours to produce a precipitate of copper thiocholine (CuThCh) at the sites of activity of the specific enzyme. The tissues of the second group were incubated in a substrate of BuThCh for 20 hours to reveal the sites of activity of the non-specific ChE. The third group of tissues was incubated without a substrate to serve as a control. The fourth group of tissues was pre-treated with DFP to inactivate the non-specific ChE and were then incubated with BuThCh (the substrate for the non-specific ChE) to serve as further controls. Following incubation the colorless precipitate of CuThCh was exposed to an ammonium sulfide solution to produce the brown staining, precipitate of copper sulfide at the sites of ChE activity. The tissues subsequently were fixed, washed, dehydrated, cleared and mounted.

RESULTS

Both specific and non-specific ChE were found to be present in the pulpal tissue of non-resorbing as well as resorbing primary teeth, Figs. 1-10. In the cuspids and molars with complete roots, relatively large amounts of acetycholinesterase were present along the neural trunks in the root canals. The smaller neural branches within the pulpal chamber were also heavily stained as were the finer neural fibers, Figs. 2 and 4. A dark, wide band of staining existed within the pulp chamber, extending along the sides and across the roof of the occlusal surface. This band along the periphery of the coronal pulp covered the area of the parietal nerve plexus and to a less extent the odontoblast area, cell free and cell-rich zones. Within the dentin, the odontoblastic processes exhibited very light if any staining. The predentin exhibited heavy staining which upon examination was found to contain needle-shaped crystals of copper thiocholine. These crystals remained after subsequent washings and thus caused a false localization at this site. This staining was evident also in the controls so it was considered an artifact. Blood vessels enveloped in fine neural elements were stained but could be distinguished from nerve trunks because of their differing structure and because the neural trunks stained much more intensely.

The non-specific ChE activity was evident in similar areas to that of the AChE but to a greater degree of intensity. Blood vessels were stained similarly to AChE except that the serum was also stained. Staining was exhibited along neural trunks and fine fibrils, Figs. 1 and 3. A band of heavy staining existed along the periphery of the pulp. The odontoblastic zone, perhaps due to the numerous small blood vessels in this area, was stained and the predentin was darkly stained.

The primary cuspids and molars with partially resorbed roots exhibited a similar pattern of ChE staining to that of the non-resorbing teeth in that nerve trunks, branches and fibers as well as blood vessels were stained, Figs. 7-10. The teeth with partially resorbed roots showed greater activity, Figs. 9 and 10,

than those with only the crown remaining, Figs. 7 and 8. The area of the parietal zone or plexus of Raschkow showed decreased staining prior to that of the large central pulpally located nerve trunks. The latter were the last recognizable structures exhibiting staining in the resorbed crowns. Light staining was exhibited in the parietal zone of some teeth with completely resorbed roots, Fig. 8, however, indicating that neural components still existed in that area. Thus the pattern of enzyme activity was not completely consistent in the resorbing tooth although in general as the extent of root resorption progressed the lighter the staining appeared. The control sections exhibited no staining except for the artifact in the predentin in group 4.

DISCUSSION

The findings of ChE in the pulp tissue of primary teeth was not unexpected because of its recorded presence in permanent human teeth, Avery and Rapp.[6] Although concentrations appear less in primary teeth, the areas of localization appear to be the same.

The non-specific ChE staining and thus the activity was noticeably more than that of the specific cholinesterase. In this regard it is of interest that Tewari and Bourne[20] found that AChE was localized in the axons while the non-specific ChE was present in the myelin sheaths. It might be reasoned that in addition to the predominance of myelinated nerves in the pulp being stained the presence of the myeline prevents the ACh enzyme from acting to reduce the substrate copper thiocholine.

The exact mechanism of pain conduction through dentin and the possible role of the odontoblast in this process is not yet known. Recently several authors have reported nerves in the dentinal tubules. Fernhead ('61),[21] Hattyasy ('62)[22] and Stella and Fuentes ('63)[23] have described unmyelinated beaded nerve fibrils in the predentin and dentinal tubules. Frank[24] most recently found structures that he believes to be nerve fibers with the electron microscope in the predentin and dentin. These findings could explain the presence of the cholinesterase found in the dentin tubules by Avery and Rapp.[6] On the other hand, Arwill ('58 and '63)[25,26] examined 2,300 electron micrographs and found no nerve fibers in the predentin or the dentin, either in the dentinal tubules or in the matrix. Rapp, et al.,[4] and Arwill ('60)[27] found nerves in the secondary predentin of older teeth and concluded they were due to advancing age or pathologic conditions or both.

Fernhead ('61)[21] studied some deciduous teeth with fully formed roots and some in which resorption had just begun. He investigated the possibility that resorption is under nervous control. Mohiuddin ('50)[28] found some degenerative neural changes preceding resorption of deciduous teeth. Fernhead[21] found similar neural elements in permanent teeth where no evidence of resorption appeared and concluded, as has Waddell[29] that a few degenerating nerve fibers can nearly always be found in a group of nerves. There is no evidence that they promote the onset of root resorption. These findings are in agreement with the present

observations as no changes in cholinesterase concentration or localization occurred prior to or during early root degeneration.

Recently Ten Cate and Shelton ('66)[30] used the technique of Karnovsky and Roots ('64)[31] for demonstration of cholinesterase and found it in nerves in the periodontal ligament and the pulp. In this technique, the thiocholine reduces copper ferricyanide to copper ferrocyanide, a colored compound. There is no need for further conversion with sulfide as in the Koelle technique. The findings of these authors are in agreement with the previous studies of Avery and Rapp[6] on permanent teeth and of the present study in deciduous teeth.

SUMMARY

Human deciduous teeth, some with complete roots and the remainder in various stages of root resorption, were studied for the presence of specific and non-specific cholinesterases. Acetylcholinesterase, the enzyme associated with acetylcholine, was found present in significant amounts in the pulp tissue of teeth with intact roots. This staining was localized along the large neural trunks and blood vessels centrally located in the pulp and in the parietal layer of nerves (plexus of Raschkow) in the periphery of this organ. The concentration of non-specific esterase appeared greater than AChE and in the same general location. The teeth undergoing resorption of the roots exhibited reduced staining which appeared proportionate to the extent of resorption.

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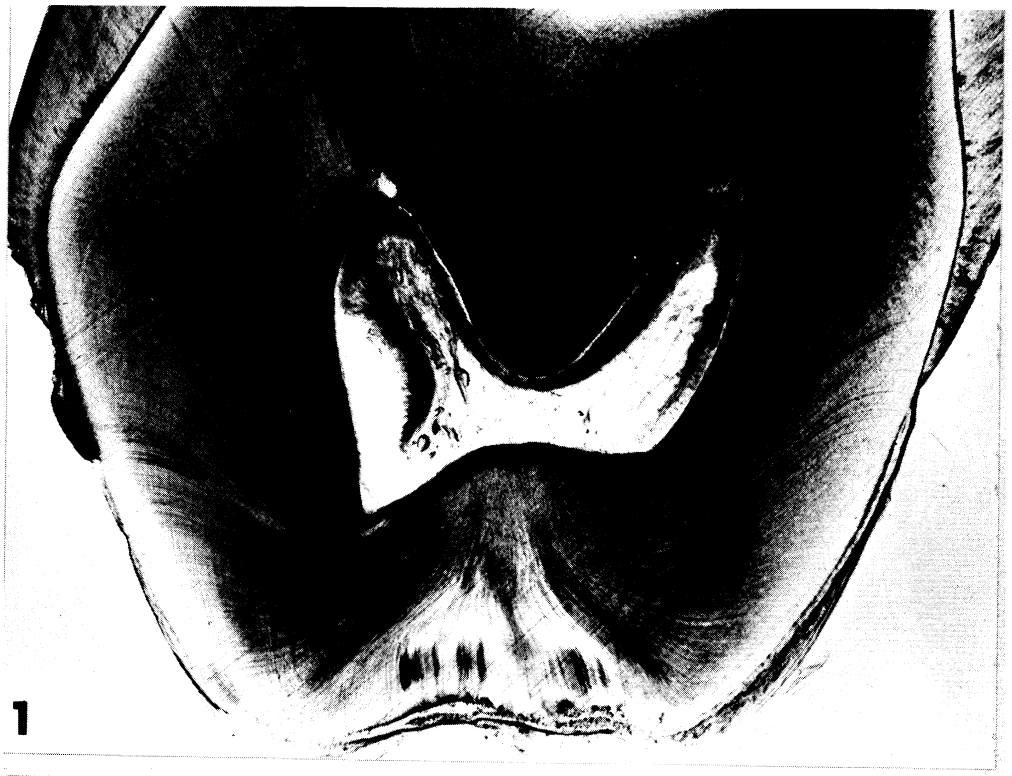
EXPLANATION OF FIGURES

Fig. 1 A calcified section of a primary molar with complete roots incubated in a substrate of BuThCh to demonstrate sites of non-specific ChE activity (Group 2). A band of intense staining is observed underlying the predentin of the pulpal chamber.

Fig. 2 A calcified section of non-resorbing primary molar treated with AThCh substrate and DFP to demonstrate centers of specific ChE activity (Group 1). A band of staining is observed in the tissue underlying the predentin in the pulpal chamber. The pattern appears similar to that of one non-specific cholinesterase but the staining intensity is less.



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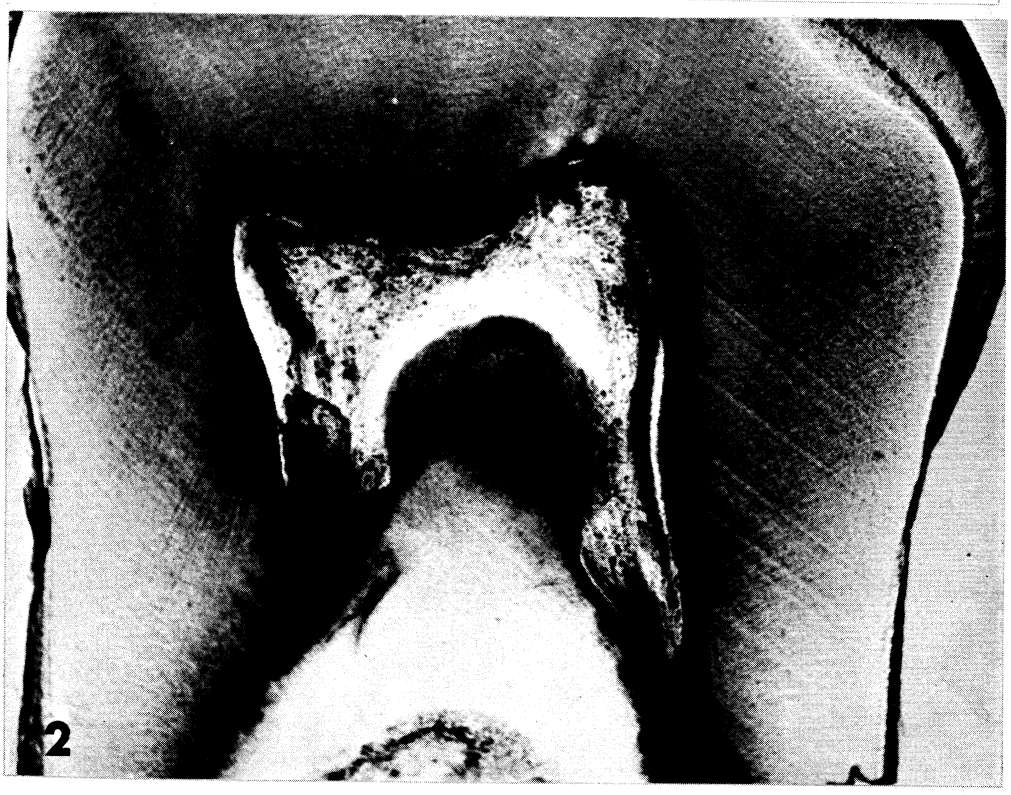

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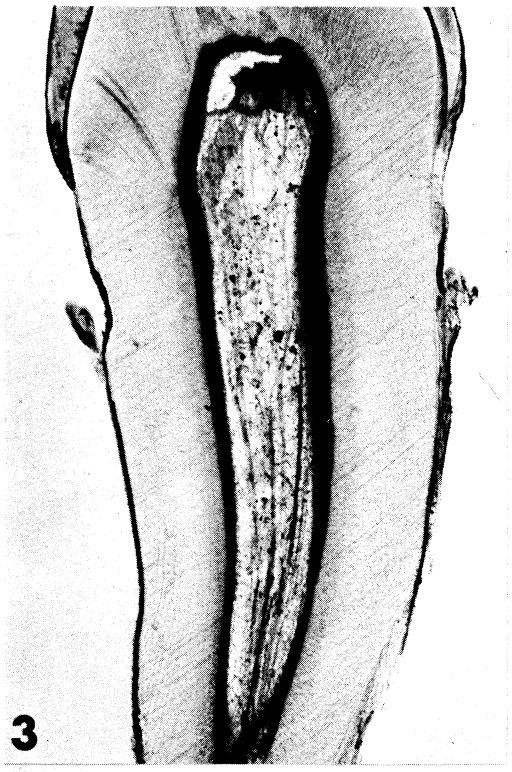
EXPLANATION OF FIGURES

Fig. 3 A section of a primary cuspid with complete roots. Sites of non-specific ChE activity (Group 2) are seen at the periphery of the coronal pulp and along blood vessels and neural trunks of the pulpal chamber and root canal. (Space at roof of pulpal chamber is an artifact).

Fig. 4 A section of a non-resorbed primary cuspid. Specific ChE activity (Group 1) found in the same locations as the non-specific ChE activity. The staining intensity of the specific ChE appears greater than the non-specific ChE but this is due to difference in section thickness. The neural elements in Figure 3 are more heavily stained.

Fig. 5 A section of a non-resorbing primary molar incubated without substrate to serve as a control. Note the absence of staining.

Fig. 6 A section of a primary cuspid treated with DFP and incubated with BuThCh substrate to serve as a control (Group 4). Note the absence of staining on the pulpal tissue.



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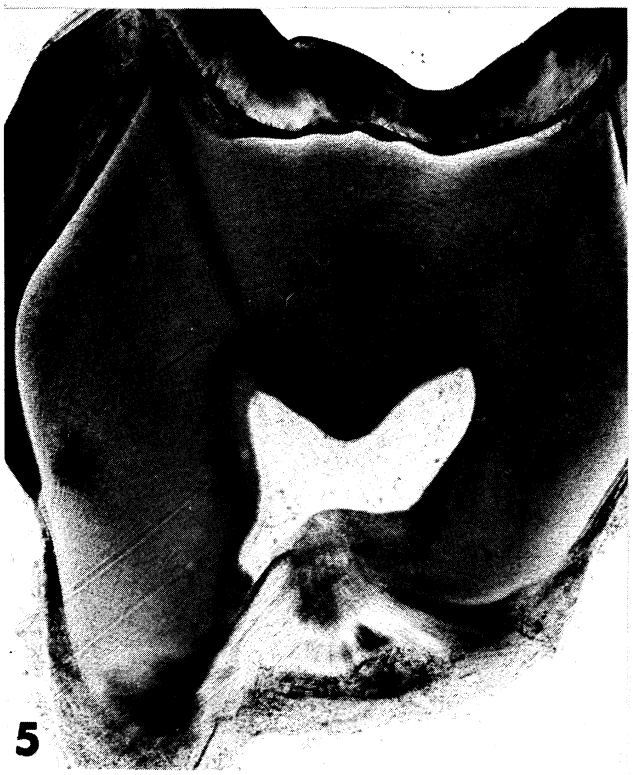

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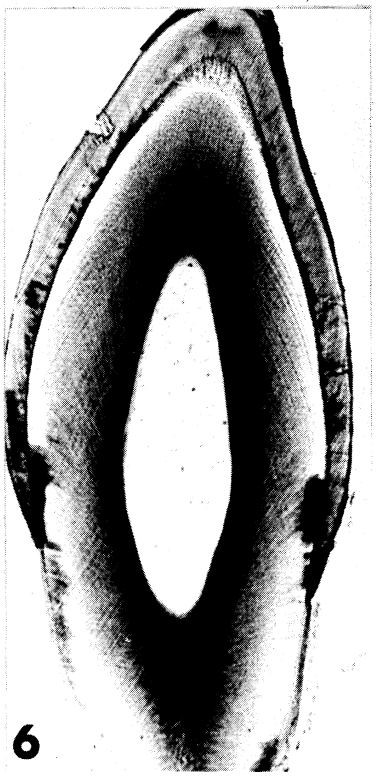

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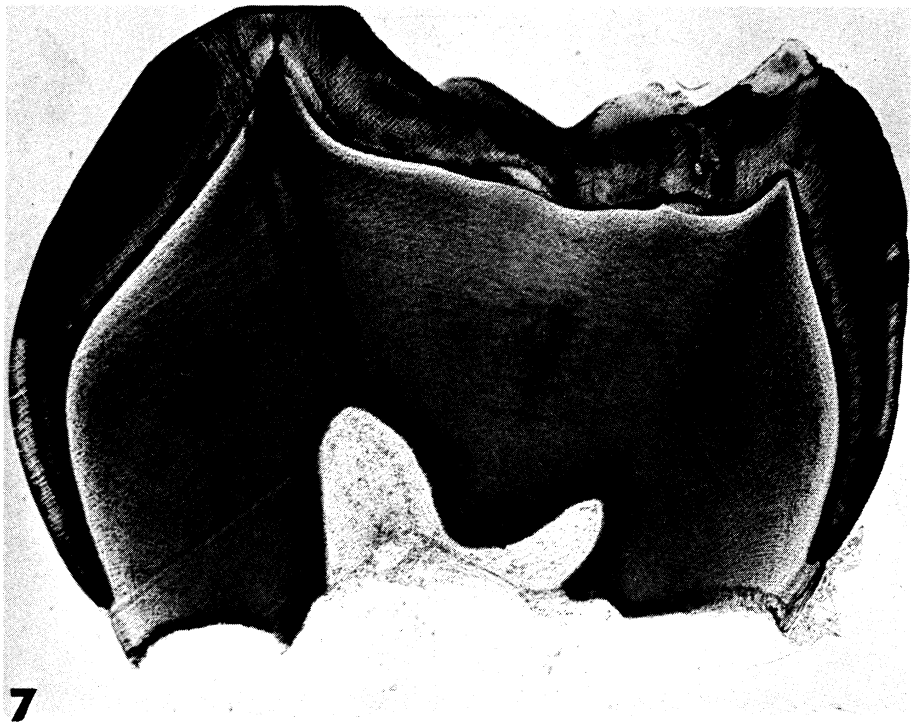
EXPLANATION OF FIGURES

Fig. 7 A section of a resorbed primary molar revealing localization of specific ChE activity (Group 1). Only the crown remains. A small amount of staining is observed in the pulpal tissue. Note the decreased amount of staining compared to the non-resorbing teeth, Figures 2 and 4.

Fig. 8 A section of a resorbed primary molar treated to show non-specific ChE activity (Group 2). The staining intensity is greater than that of Fig. 7 but considerably less than that of non-resorbing teeth, Figs. 1 and 3.



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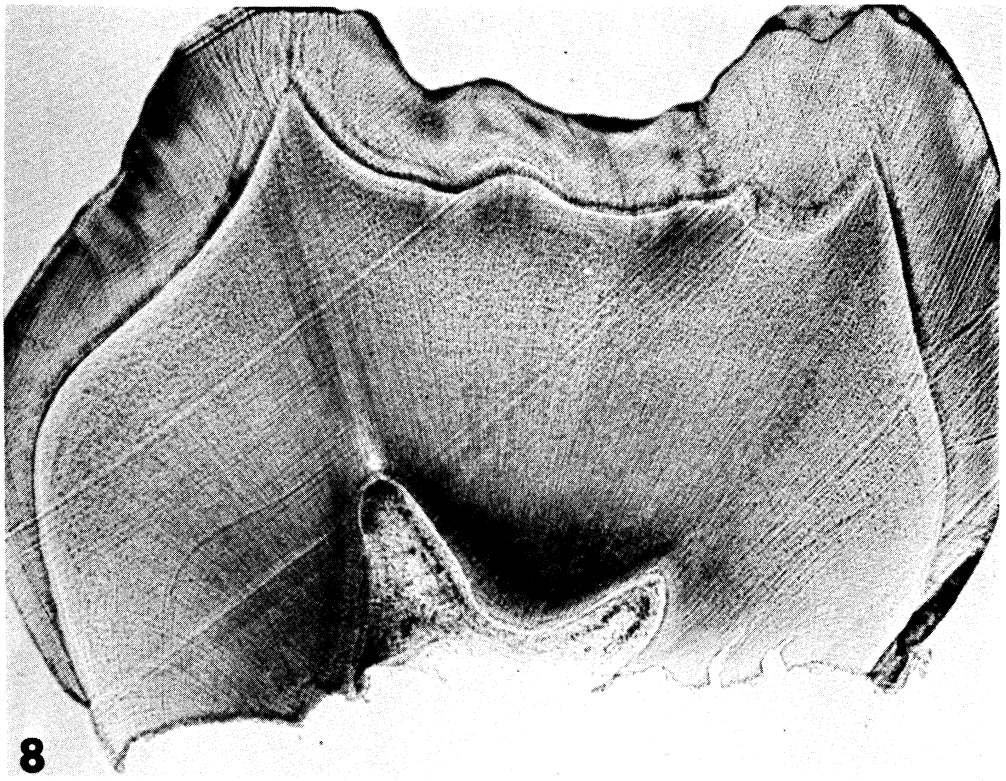

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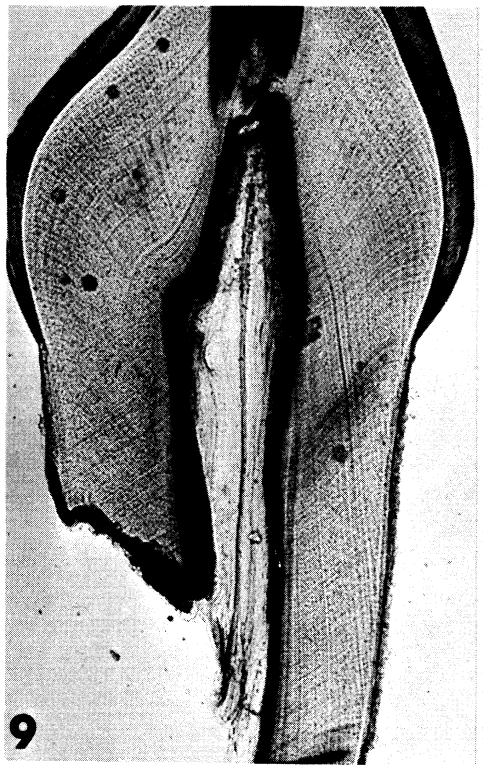
EXPLANATION OF FIGURES

Fig. 9 A calcified section of a resorbing primary cuspid in which the roots are approximately one-half missing. Sites of activity of non-specific ChE staining (Group 2) appear along nerve trunks and blood vessels in the crown and roots especially along the periphery of the pulp chamber.

Fig. 10 Sites of specific ChE staining (Group 1) are seen along periphery of pulpal chamber and to some extent along the nerve trunks and blood vessels. The staining is more evident than that of the completely resorbed root, Fig. 7.

Fig. 11 A section of a resorbed molar in which the roots are resorbed to the crown of the tooth. The section is treated with DFP to inactivate the non-specific cholinesterase and incubated with butyryl-thiocholine to serve as a control. Note the complete absence of staining in the pulpal tissue.



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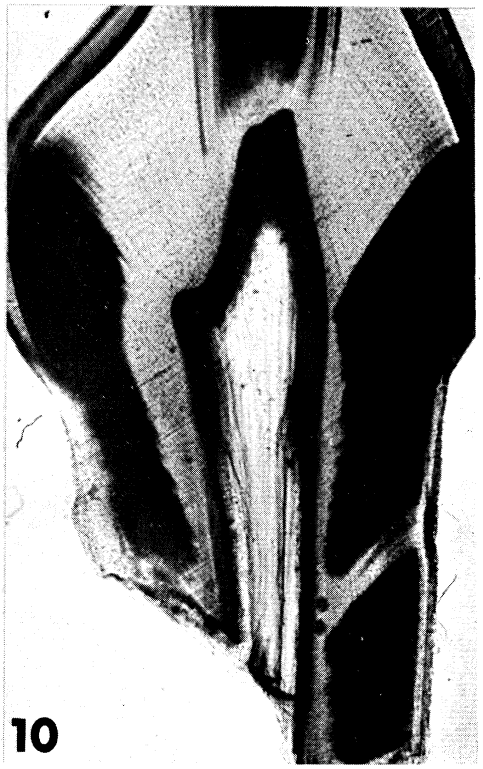

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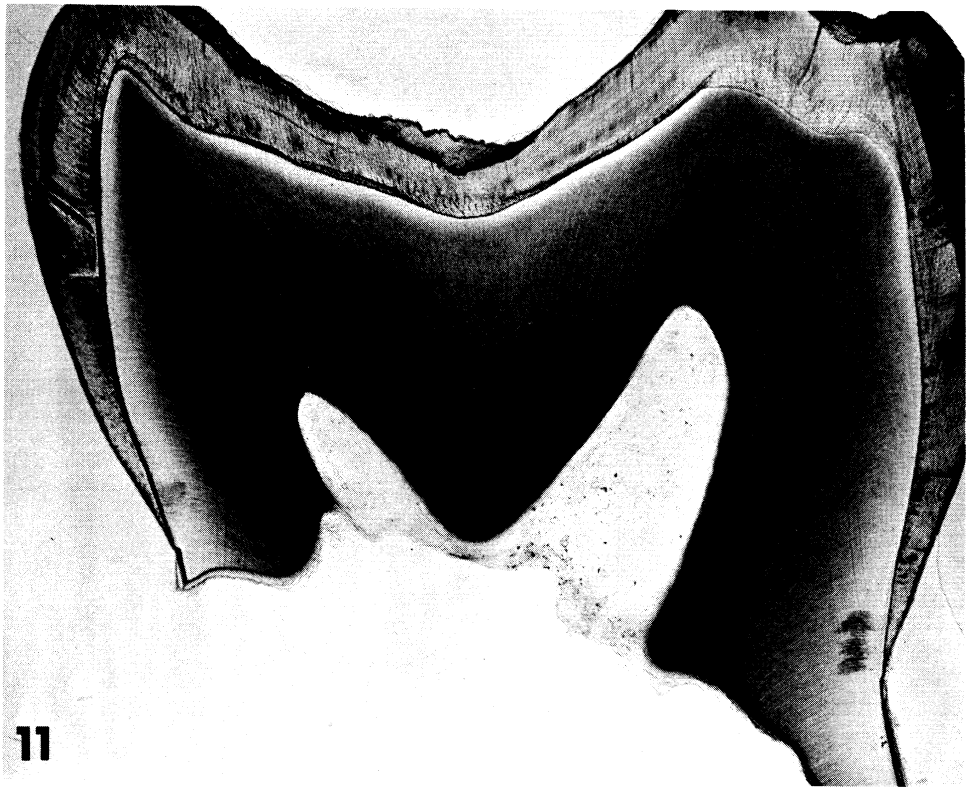

11

Distribution of Nerves in Human Primary Teeth

The innervation of human primary teeth has received little attention when compared to similar research on permanent teeth. The lack of interest in the distribution of nerves in the primary dentition may be because the pulps are believed to be similar to that of permanent dentition or that they are not a lasting component of the human being. Clinicians have long been aware of a lower sensitivity of primary teeth as compared to permanent teeth. The differences in the sensitivity of these teeth may be due to differences in number and/or distribution of the neural components within them. Another area in which little is known is the changes in the neural tissue in the primary teeth during the period of exfoliation and root resorption. The present investigation is concerned, therefore, with the distribution of nerves in primary teeth during stages of root formation, root completion and root resorption.

Bradlaw (1936) studied microscopically neural degenerative changes of both human and animal primary teeth using a variety of silver impregnation techniques. He observed these changes to occur prior to exfoliation in some teeth while not in others. Mohuiddin (1950) studied the primary and permanent teeth of the cat by means of both the Romanes' and Bielschowsky silver method. The permanent and non-resorbing primary teeth exhibited patterns of innervation similar to that observed for human permanent teeth by Rapp, Avery and Rector (1951). All of these investigations observed neural fibers within the pulp to form an arborization or parietal layer, (plexus of Raschkow) adjacent to the dentin and then terminate between or among the odontoblasts. A few nerves were found in the predentin and dentin, but none were observed coursing within the dentinal tubules. Mohuiddin was interested in whether neural changes preceded or followed the onset of pulpal disorganization in exfoliation. He found some degenerative neural changes appear prior to evidence of root resorption. He noted degenerative changes were limited to nerves one micron or more in diameter. These changes were characterized by varicosities and vesicular formations and fragmentation within the nerve fibers. They were not observed in every nerve fiber or in every fiber with a nerve bundle. As resorption advances, however, the number of neural fibers in the pulp decreases until only occasional fragments of axons of a fine caliber can be seen.

Fernhead and Linder (1956), and Fernhead (1961) used silver impregnation techniques to study developing and resorbing human primary teeth. Fernhead noted a marked increase of small diameter nerve fibers in the pulps during root formation. The plexiform arrangement of fibers was not evident in the deciduous teeth at as early a stage as in the permanent. His observations of the organization of neural fibers in permanent teeth corresponded to those described by Rapp, Avery and Rector (1957). Fernhead observed a marginal plexus of fibers formed adjacent to the predentin from which individual fibers became embedded in the

predentin while others entered the dentinal tubules and were closely associated with the odontoblastic process. He found some nerve fibers to have undergone degenerative changes in both deciduous and permanent teeth. The later showed no evidence of root resorption. He concluded that degenerating nerve fibers can usually be found in groups of otherwise normal preterminal nerves and thus there is no evidence that primary tooth root resorption is under nervous control.

MATERIALS AND METHODS

Seventy-five human primary teeth in the stages of root formation, completion and various stages of resorption were studied. Immediate fixation was carried out in a ten per cent solution of neutral formalin containing ten per cent chloral hydrate followed by decalcification in three per cent hydrochloric acid. The teeth were embedded in paraffin and sectioned at thicknesses ranging from ten to 30 micra. Powers' (1952) modification of the Romanes' silver impregnation technique was used to demonstrate neural tissue.

RESULTS

A. Primary Teeth with Completed or Nearly Completed Roots

Nerve trunks containing many individual, myelinated, nerve fibers pass through the apical foramen of the tooth laterally directed into the root canal (Fig. 1). Within the root canal only an occasional branch rises from these trunks, (Fig. 2). Many of the nerve trunks within the root canal were found to exist independent of vessels (Fig. 1), while others were found in close association with blood vessels (Figs. 2,3).

The nerve trunks pass into the coronal pulp chamber and then divide into smaller branches which divide further as they pass laterally towards the dentin walls and into the pulpal horns (Fig. 4). These smaller nerve fibers divide and eventually become single fibers which interweave to form the parietal network of nerves (plexus of Raschkow), (Figs. 5,6). This plexus, located along the periphery of the pulp chamber, is composed of both myelinated and unmyelinated fibers adjacent to the cell-rich zone (Figs. 5,6). An occasional single, unmyelinated fiber arises from this parietal plexus and passes through the cell-rich zone and cell-free zone (Zone of Weil) to terminate among the odontoblast cells, (Figs. 6,7). No specialized endings or attachments are observed between nerve fibers and the odontoblasts. Nerves are not observed to penetrate the substance of the predentin or dentin. The pattern of distribution of neural elements in the forming and completed primary teeth appears to be very similar to that of young permanent teeth.

B. Primary Teeth with Resorbing Roots

Primary teeth that have undergone early stages of root resorption display little if any change in the pattern of neural distribution from that seen in the nonresorbed teeth. A few signs of neural degeneration appearing in the form of varicosities and vacuoles were seen in a few of the nerve fibers (Figs. 8,9 and 10). However, in teeth with approximately half of the root dentin resorbed, the majority of the fibers exhibit varicosities and vacuole formation. In addition, fragmentation of some of the nerve fibers was observed, as well as a general decrease in the total amount of neural tissue present within the tooth (Fig. 10). Some show progressive degeneration with progressive root resorption while others show little correlation in the amount of neural resorption and degeneration. Teeth with only the crown dentin remaining exhibit a varied pattern of innervation of the pulp organ, (Figs. 11, 12 and 13). In some instances, a nerve trunk persists in which there is loss of continuity of the fibers, (Fig. 11). The number of individual fibers within the trunk appears to be decreased in some cases when compared to those seen before onset of root resorption (Fig. 13). In other teeth an occasional nerve fiber or groups of fibers or the remnants of a parietal plexus of nerves is seen (Fig. 12). On the other hand some completely resorbed teeth show a complete absence of nerves.

Root resorption is seen often to begin on the sides of the root rather than at the apical end (Fig. 14). In such instances, signs of inflammation may be visible within the pulpal tissue opposite the resorption site. Nerves passing through this area persist in spite of the resorption and inflammatory process. Teeth with nearly completely or completely resorbed roots exhibit similar signs of inflammation in the pulp.

The character of the pulpal cells appears to change as root resorption becomes advanced, as they become separated or decreased in number and assume an embryonic-like appearance (Fig. 13).

DISCUSSION

Fernhead noted an increase in neural components between onset of dentinogenesis and completion of root development. He further observed that a plexus of Raschkow is not present in primary teeth by the time of root completion. This is neither substantiated by Mohuiddin or by this present study in which this structure is seen during this latter phase of development. Teeth in the early stages of root formation were not included in this investigation however. Fernhead further stated that it is impossible to compare patterns and density of innervation of primary and permanent teeth at similar stages of development without taking into consideration the fact that permanent teeth require six to nine years between dentinogenesis and root completion as compared to 12 to 16 months for primary teeth. This difference in maturation time between primary and permanent teeth does not mean that a primary tooth

at root completion cannot be innervated to the same or similar extent as a permanent tooth at a similar stage of development. Evidence for this is supplied by the observations in this study, in which a similar pattern of innervation existed at similar stages of development in the primary and permanent teeth.

There appears to be agreement by Mohuiddin, Fernhead, Rapp, Avery and Rector that the general pattern of innervation of both primary and permanent teeth after root resorption had begun, is similar. In no instance, however, were nerves seen to enter the calcified tissues of primary teeth in the present study. Numerous authors, Bernick and Rapp and Avery have noted neural fibers in predentin and dentin of permanent teeth, however. Mohuiddin describes some nerves passing into the predentin of the cat while most appear to terminate about the odontoblasts. Rapp, Avery and Rector have attributed nerves in the predentin matrix to be a result of entrapment during its deposition. Fernhead describes nerve fibers entering dentinal tubules and recently several investigators have reported nerve fibrils in the dentin. Hattyasy (1961), and Stella and Fuentes (1961) have described intratubular nerve fibers with the light microscope and recently Frank reported the presence of such fibrils in dentin. Arwill on the other hand studied the dentin extensively with the electron microscope and found no nerve fibers.

Mohuiddin again observed unusual changes in the nerves of deciduous teeth and attributed them to be degenerative in nature. Fernhead was of the opinion that similarly appearing changes in nerves were due to incomplete fixation. Changes similar to those described by Mohuiddin were observed in the present study. Thickening of the fibers (varicosities), thinning of these enlarged areas (vaculations) and fragmentation were observed. These were frequently seen following the onset of root resorption although rarely before this stage. Similar changes were observed in the teeth of vitamin A deficient rats by King, Lewinsky and Stewart (1938) and were considered to be evidence of neural degeneration. Again, similar appearing changes in nerves of frog tadpoles have been described by Speidel (1936), and by Torrey (1936) of the taste buds of catfish and Wedell and Glees (1941) on nerves of rabbit cutaneous tissue. As such changes within neural tissue of teeth are not observed in nonresorbing primary teeth or in permanent teeth, their presence is probably not due to such factors as incomplete fixation.

The earliest and most frequently observed degenerative change seen was of the thickening of the neural fiber and the production of a varicosity. With progressive root resorption formation of vesicles and fragmentation of the fibers was seen. This led to a general reduction in total neural density. Degenerative changes were not found in all fibers in the pulp organ or in all fibers within a single neural trunk.

Mohuiddin described degenerative changes to begin in pulp nerves before the onset of root resorption. The observations of this study would suggest that

such changes follow and occur as a result of onset of root resorption. Observations of this nature must be speculative as it is difficult to determine the exact time of initiation of resorption. Again many subtle biochemical, if not morphologic, changes may be occurring in the pulp prior to noticeable alteration of the root surface. Observations of this study of a reduction in amount of neural fibers in the human pulp organ with progressive resorption are in accordance with the findings of Mohuiddin in the cat.

Finally, the pattern of distribution of nerves in the nonresorbing human primary tooth is similar to that described in the permanent teeth by Rapp, Avery and Rector. The appearance of the large neural trunks in the central pulp region and the parietal layer peripherally were a common characteristic. The density of the innervation of the primary tooth is not as great as that of the permanent tooth, however. The plexus of Raschkow of the primary tooth does not consist of as dense a network of myelinated fibers as does the counterpart in the permanent tooth. In addition, fewer fibers are seen to arise from this neural network to pass to the odontoblasts in primary teeth as compared to permanent teeth. This is possibly the reason that primary teeth are less sensitive to operative procedures than are permanent teeth.

ABSTRACT

Human primary teeth in the stages of late root formation, root completion and root resorption were impregnated with silver to allow study of the distribution of nerves in the pulp.

Neural trunks composed of many individual, myelinated nerve fibers enter the apical foramen of the tooth and pass in a coronal direction, often in close association with blood vessels. Neural trunks in the crown of the tooth divide to smaller branches which continue to divide to myelinated nerve fibers which become interwoven to form the parietal layer (plexus of Raschkow). The occasional unmyelinated fiber leaves this zone and passes into the odontoblastic layer of cells where it appears to terminate with no apparent specialized ending. No nerves were seen to enter the predentin or dentin.

As primary teeth began to undergo root resorption, degenerative changes appeared in the nerves such as thickenings, varicosities and fragmentation and the quantity of neural tissue decreased. The greater the amount of resorption, the greater were the degenerative changes. Teeth in which the roots were almost completely resorbed showed only a sparse number of nerves present.

The pattern of distribution of nerves in primary teeth was observed to be similar to that of permanent teeth. The density of the innervation of the primary teeth, however, was not found to be as great as that of permanent teeth.

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The following photomicrographs illustrate sections of human primary cuspids and molars impregnated with silver to delineate neural tissue. Figures 1-8 show teeth in which no root resorption is occurring while Figs. 9-14 show teeth undergoing root resorption.

Key to Abbreviations in Figures

B.V.	-	blood vessels
C.F.	-	cell-free zone (Zone of Weil)
C.R.	-	cell-rich zone
D.	-	dentin
I.C.	-	inflammatory cells
N.	-	nerve
N.N	-	parietal network of nerves (Plexus of Raschow)
N.T.	-	nerve trunks
O.	-	odontoblasts
P.	-	pulp
R.R.	-	root resorption

Fig. 1 Area of apical foramen of a developing primary molar. Several nerve trunks consisting of many myelinated nerve fibers enter the open foramen and pass in a coronal direction. The pulp tissue is highly cellular in this the proliferation zone.

Fig. 2 Mid-length region of the root canal of a primary cuspid not undergoing root resorption. Nerve trunks pass in a coronal direction with a minor amount of branching and often in close association with blood vessels.

Fig. 3 Root canal of a completely formed primary cuspid. Many individual, myelinated, nerve fibers compose these centrally located nerve trunks. No signs of neural degeneration is evident.

Fig. 4 Pulp chamber of a nonresorbed primary cuspid. Nerve trunks pass from the root canal into the coronal pulp chamber where the divide into smaller branches.

1

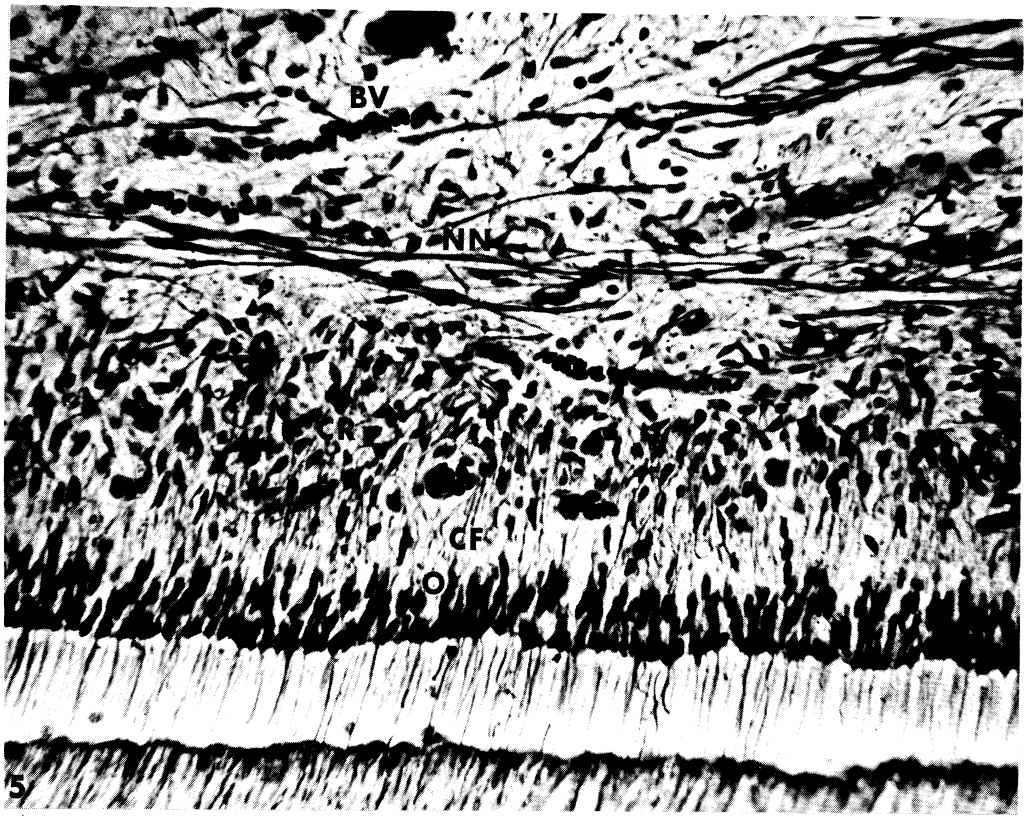
2

3

4

Fig. 5 Pulp chamber of a nonresorbed primary cuspid. Individual, myelinated, nerve fibers pass peripherally in the pulp organ to form the parietal network of nerves (plexus of Raschkow). Note the cell-rich (CR) zone adjacent to the neural network and below the cell-free (CF) zone lies adjacent to the odontoblasts. Many blood vessels containing blood cells are evident in the peripheral area of the pulp.

Fig. 6 Pulp chamber of a nonresorbed primary cuspid. Nonmyelinated nerve fibers from the parietal network pass through the cell-rich and cell-free zones to terminate without special endings among the odontoblasts.



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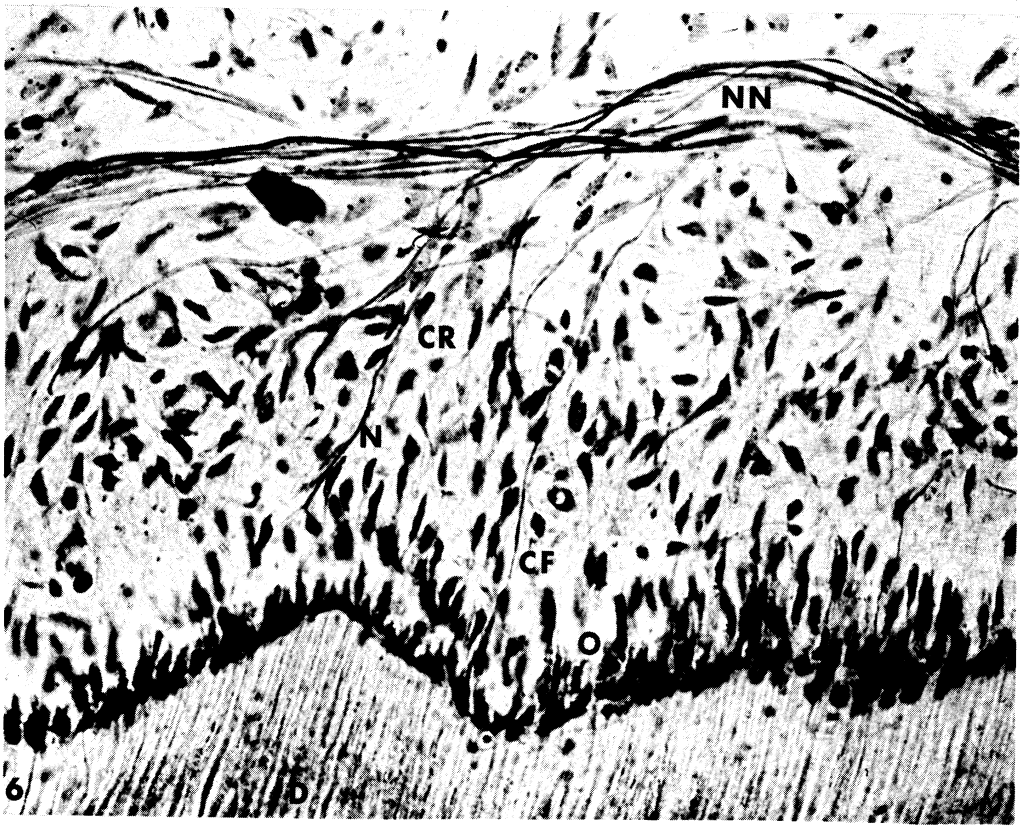

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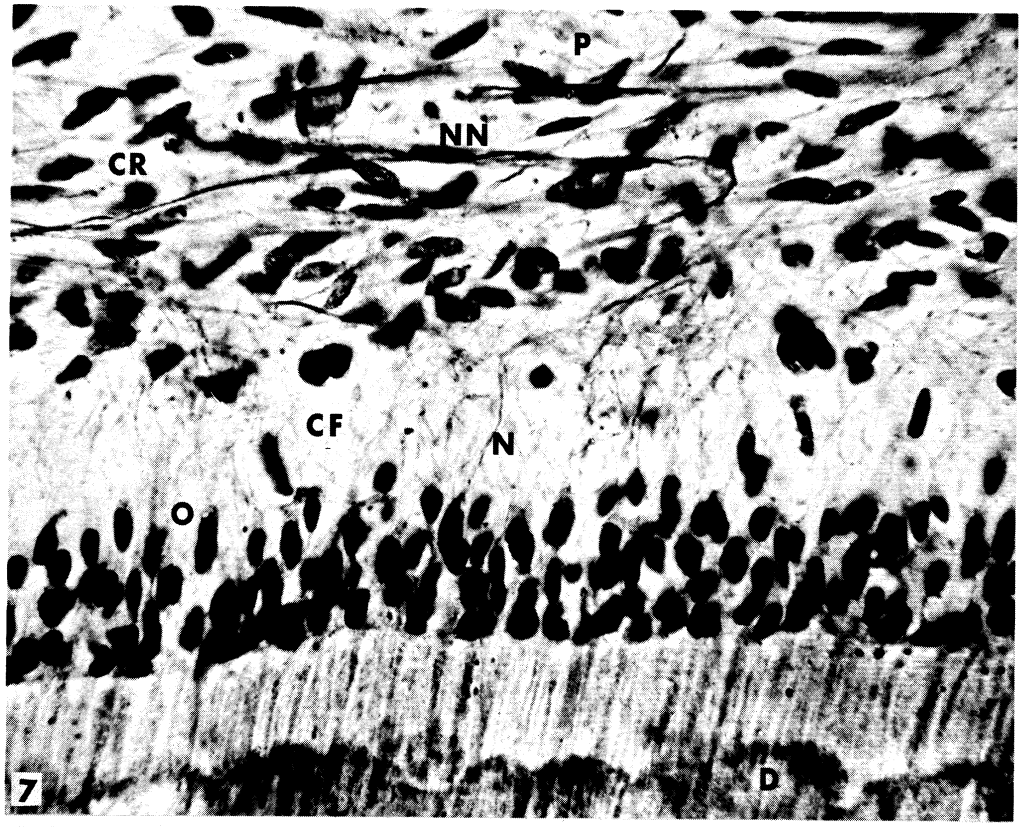
Fig. 7 Pulp chamber of a nonresorbed primary molar. A nonmyelinated nerve fiber passes from the parietal network through the cell-rich and cell-free zones to the darkly stained odontoblastic layer (O).

Fig. 8 Pulp chamber of a resorbing primary molar. A high magnification of a nerve trunk and a blood vessel illustrating the close relationship of the two. Note evidence of early degenerative changes in the nerve fibers.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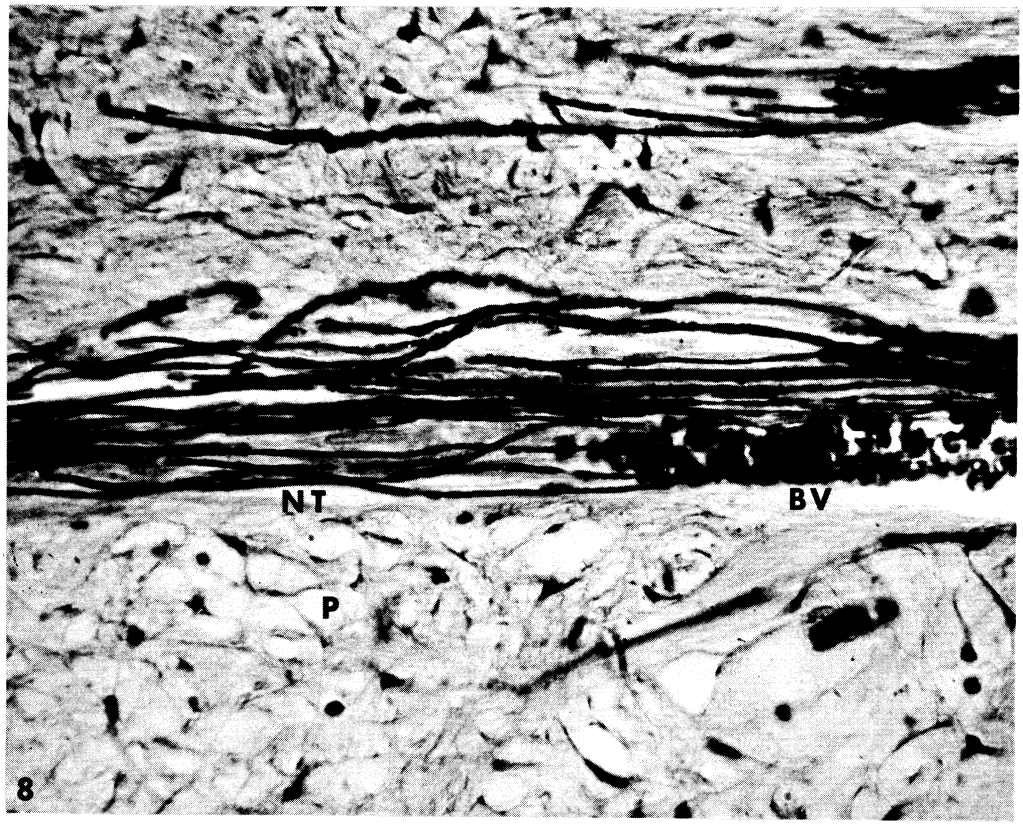
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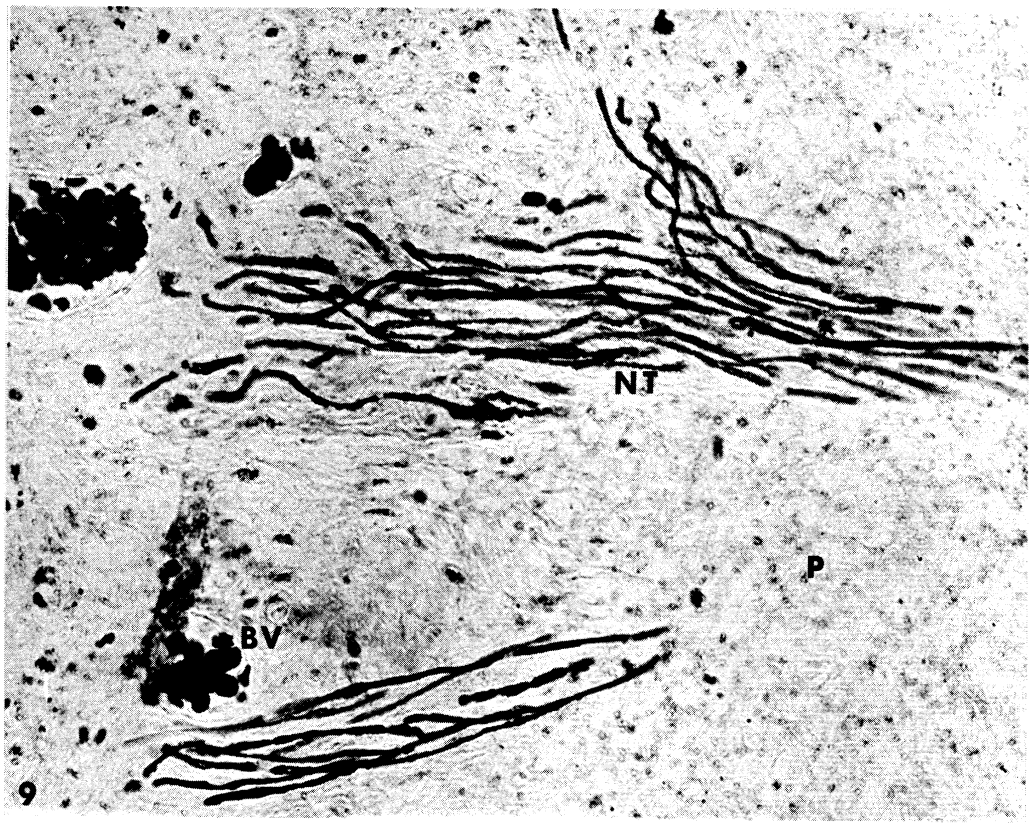
Fig. 9 Pulp of a primary cuspid undergoing resorption. A high magnification of nerve branches illustrating degenerative changes which occur upon initiation of root resorption. Varicosities and vacuolation are evident in the nerve fibers.

Fig. 10 Pulp of a primary cuspid undergoing resorption. Degenerative changes in the form of varicosities and vacuolation are observed in the nerve tissue.



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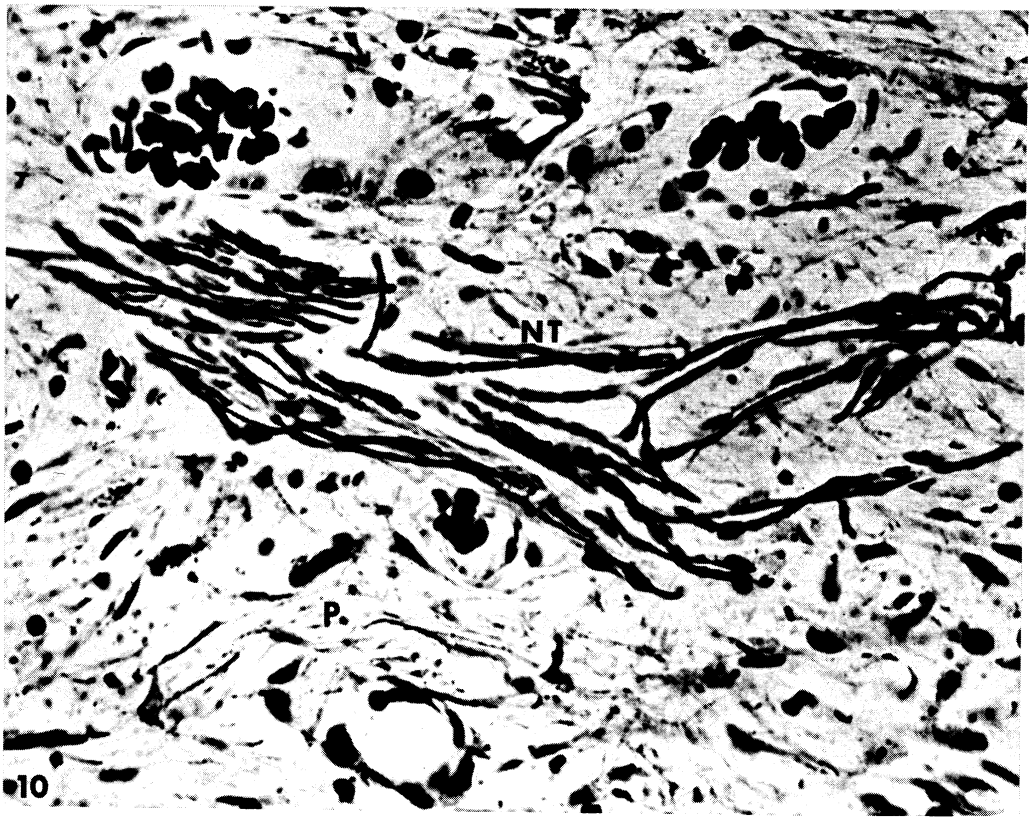

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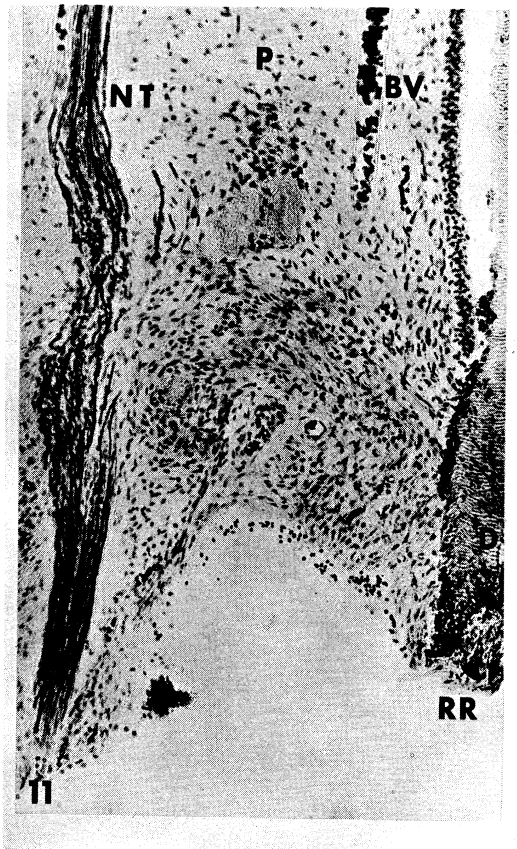
Fig. 11 Pulp chamber of a resorbing primary cuspid. A nerve trunk still persists in the pulp following nearly complete resorption of the root dentin. Continuity of the fibers within the trunk appears to be interrupted and the fibers decreased in number. Note the absence of smaller nerve branches or individual fibers. Degenerative signs are present within the nerve tissues as well as evidence of the cellular infiltration of inflammation within the pulp.

Fig. 12 Crown of a primary cuspid undergoing root resorption. Several nerve fibers persist although the root dentin of the tooth has been nearly completely resorbed. The neural organization of the nerves seen in nonresorbing teeth is absent and the total neural content decreased.

Fig. 13 Crown of a primary cuspid undergoing root resorption. The amount of nerve tissue present in this tooth is less than seen in teeth of Figs. 9 and 10 although the amount of root resorption is the same. The structure of the pulp tissue is altered having become more cellular in nature.

Fig. 14 Root of a primary cuspid undergoing root resorption. Resorption is taking place along the side of the tooth rather than at the apical area. Nerve trunks and branches persist in spite of an inflammatory reaction that appears in response to the process.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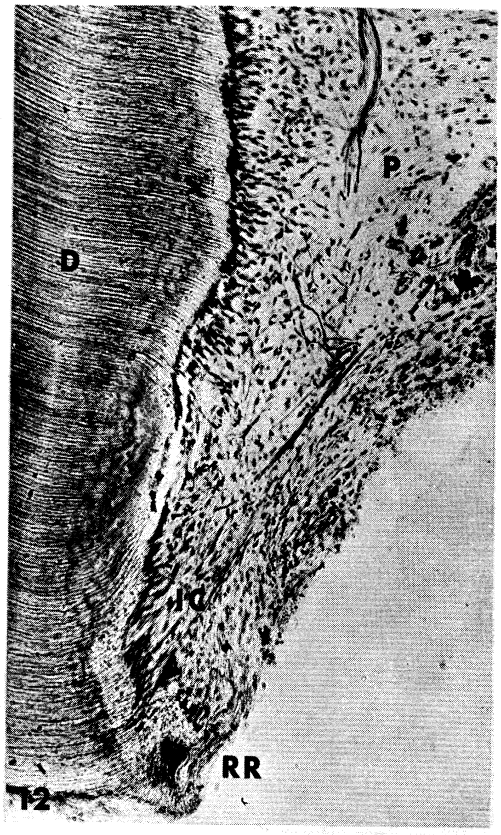
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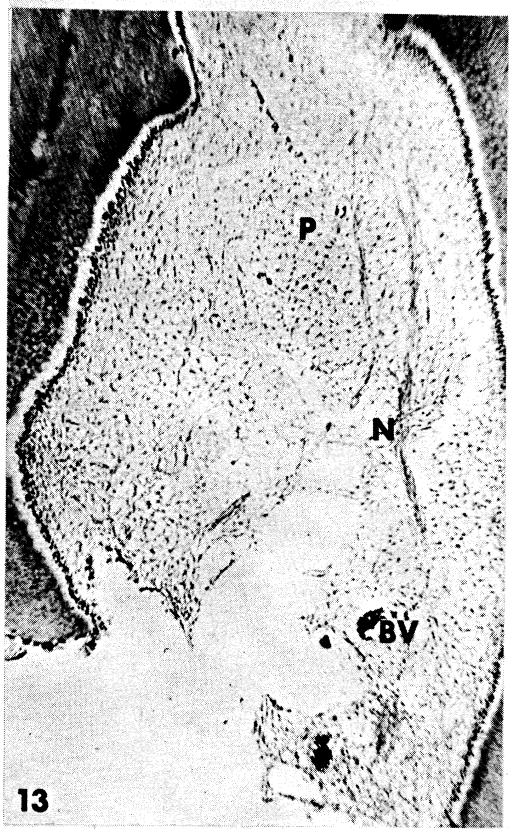
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Esterases In The Human Pulp As Determined By Gel Electrophoresis

With the refinement of electrophoretic techniques, many investigators have attempted to separate and identify enzyme constituents of various tissues. Burstone[1] has compiled numerous histochemical techniques usually applied to tissue sections but which also find applications in staining gels after electrophoretic separation. Avery and Rapp[2] have applied the thiocholine technique of Koelle[3] to demonstrate acetylcholinesterase (AChE) and non-specific cholinesterase (ChE) in human primary and permanent tooth pulps. Recently Ten Cate and Shelton[4] used the technique of Karnovsky and Roots[5] for demonstrating ChE and found it present in nerves of the periodontal ligament and the tooth pulp. In this technique the thiocholine reduces copper ferricyanide to copper ferrocyanide, a colored compound. There is no need for further conversion with sulfide as in the Koelle technique. They have found, as have Avery and Rapp, ChE along nerve trunks in the pulps of permanent teeth, although they found none in the odontoblast and its process.

Avery and Rapp[2] have related AChE activity and its localization in the tooth pulp to the mechanism of pain conduction in the pulp. This is based on the fact that AChE is associated with acetylcholine, the neurohumeral transmitter found along nerves and especially at synapses.

Hunter and Markert (1957)[6] and Hunter and Strachan (1961)[7] pioneered in the demonstration of esterases on starch gels. Their technique was applied to human tooth pulps, and in an attempt to increase the resolution of these esterase patterns, it was decided to electrophorese the pulps on acrylamide gels according to the technique of Ornstein and Davis.[8] The purpose of the present study therefore is to determine the number and types of esterases that can be electrophoretically separated on these two gel media.

MATERIALS AND METHODS

Approximately 200 human permanent teeth were cracked open and the pulps were removed and weighed. These were then homogenized in 40% sucrose (0.01 ml/mg of pulp tissue); the homogenization was carried out in ice water. In starch gels, the electrophoretic technique was that of Hunter and Markert[6] with minor modifications. In acrylamide gels, the electrophoretic technique of Ornstein and Davis was used, but modified somewhat to prevent denaturization of the esterases which might occur by incorporating them in a sample gel polymerized at room temperature. By homogenizing the pulps in 40% sucrose, centrifuging the mixture and layering the supernatant directly onto the spacer gel, maximum esterase activity was preserved. The homogenate of 15 mg of pulp tissue was added to each gel. The acrylamide gel electrophoresis was carried

out at 4°C with a current of 1.5 mamp/gel.

Both the starch and acrylamide gels were then stained in the same types of substrate solutions to demonstrate the esterases present. These consisted of:

2.5×10^{-4} M α-naphthyl acetate or α-naphthyl butyrate

0.1 M phosphate buffer at pH 7.5

1 mg of Blue RR diazonium salt per ml of solution

A modification of the Koelle technique[3] was applied to some of the gels to demonstrate acetylcholinesterase (AChE) and cholinesterase (ChE). In addition, inhibition studies were carried out using DFP and eserine sulfate in an attempt to identify the bands observed. These were included in the substrate incubation solutions at concentrations of 10^{-5} M. (Several concentrations were tried, but 10^{-5} M proved adequate.)

RESULTS

Whereas starch gels resolved three esterase bands, the acrylamide gel electrophoresis demonstrated four. The fourth band was nearer the origin than the others (the cathodic end of the gel). This fourth band (not seen in starch gels) was the only one stained by the Koelle technique. Its sensitivity to inhibition by DFP and eserine sulfate indicates that it is a cholinesterase, but whether or not it is acetylcholinesterase cannot be determined from this data. This fourth band also appeared in electrophoretic separations of whole homogenized blood, in which case the staining was much darker. Bands two and three of tooth pulps as seen in acrylamide gels stained with α-naphthyl acetate or α-naphthyl butyrate, did not appear to any extent in the blood sample gels.

SUMMARY

Band four as seen in the acrylamide gels would seem to be a cholinesterase because of its following properties:

1. Electrophoretic mobility
2. Reaction with acetylthiocholine and butyrylthiocholine
3. Sensitivity to DFP and eserine
4. Presence in blood as well as pulp tissue.

The relationship of the ChE to neural elements and not only to the blood would have to be demonstrated by comparing its activity before and after a nerve resection experiment, which is planned. The failure of this esterase band four to migrate into starch gel could be due to a difference of the pore sizes be-

tween the starch and acrylamide gels.

Bands three and two represent esterases found in very small quantities in the blood, but in appreciable amounts in the pulp tissue. While neither is a ChE, they both are inhibited considerably by DFP. Since band four is inhibited by DFP and eserine, a histologic study using incubating solutions of α-naphthyl acetate or α-naphthyl butyrate containing eserine might localize the esterases of bands three and two in the pulp. Tissue sections can be obtained by slicing the teeth on a calcified tissue slicing machine or by sectioning the frozen pulps on a cryostat. In addition better localization of AChE in acrylamide gels may be obtained using a technique devised by Davis[9] (modified from a technique described in Pearse's Histochemistry and from L. L. Uzman's, Lab. Invest., 5:229, 1956).

Band one appeared in samples of both tooth pulp and blood. This esterase reacted much more strongly with the α-naphthyl acetate.

Electrophoresis of tissues rich with AChE (brain, red blood cells and cervical sympathetic ganglion) will help localize the position of AChE in the gel. Similarly electrophoresis of liver and serum will help localize the pseudocholinesterases.

Experiments are in progress to further quantitatively evaluate the AChE activity in the pulp tissue using a pH-stat.

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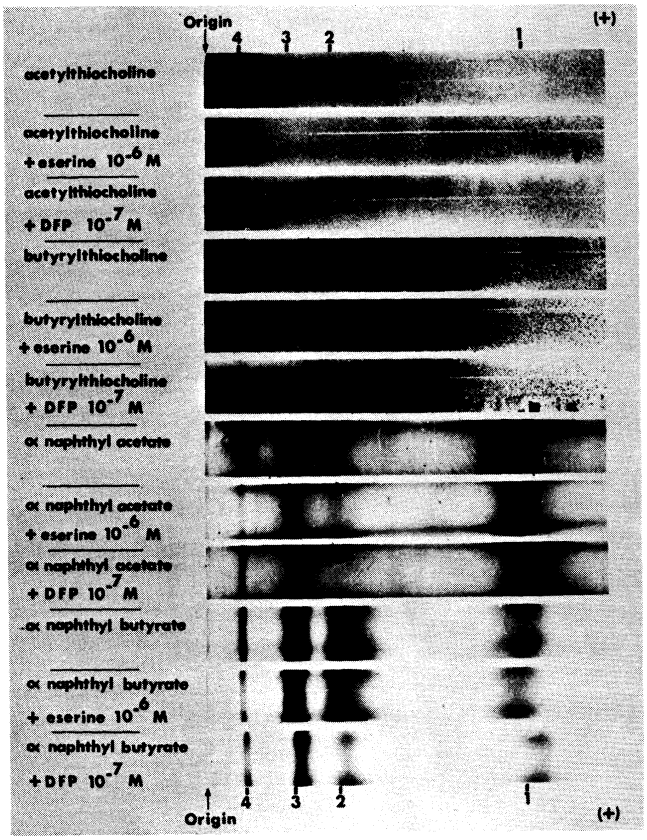
Upper Figure

Acrylamide gels of human dental pulp stained with the substrates noted to the left of each gel. Inhibitors and their concentrations are also noted. The bands are numbered one to four from the anode to cathode ends of the gels. Four bands are shown using the naphtholic substrates and one band (band four) is shown using thiocholine substrates. Note that band four is inhibited by DFP and eserine in both thiocholine and naphtholic substrate solutions, while bands two and three of pulp tissue are inhibited significantly by DFP alone.

Lower Figure

Acrylamide gels of human dental pulps and whole, homogenized blood stained with the substrates noted to the left of each gel. Bands one and four of human pulp correlate with two of the bands shown in whole blood. Bands two and three of tooth pulp do not stain significantly in whole blood samples. Thus, bands two and three are associated with the tissue of the pulp. Comparison studies with other body tissues would have to be made to determine whether they represent esterases unique to the pulp.


Origin
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4
3
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1
acetylthiocholine
acetylthiocholine + eserine 10^{-6} M
acetylthiocholine + DFP 10^{-7} M
butyrylthiocholine
butyrylthiocholine + eserine 10^{-6} M
butyrylthiocholine + DFP 10^{-7} M
α naphthyl acetate
α naphthyl acetate + eserine 10^{-6} M
α naphthyl acetate + DFP 10^{-7} M
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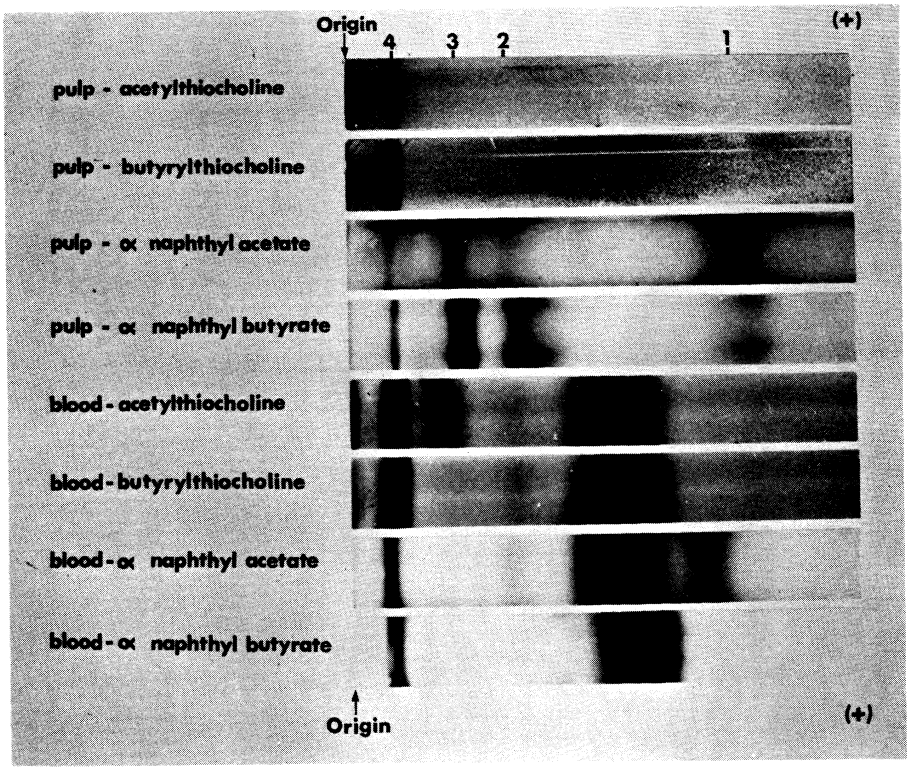
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pulp - acetylthiocholine
pulp - butyrylthiocholine
pulp - α naphthyl acetate
pulp - α naphthyl butyrate
blood - acetylthiocholine
blood - butyrylthiocholine
blood - α naphthyl acetate
blood - α naphthyl butyrate
Origin
(+)

Pulpal Alteration in Nerve Resection

Several investigators have reported the effects of denervation of the teeth on the anatomy of the pulp tissue. King[1] resected the cervical sympathetics in rabbits and found a temporary acceleration of growth of the incisors which he attributed to vasodilatation. Taylor and Butcher[2] found a 20-30% increase in the eruption rate of rat incisors after an inferior alveolar nerve resection.[2,3] They concluded this lack of sensation caused tooth fracture which resulted in lack of occulsion which in turn possibly resulted in increased eruption. They did not observe any effect on the development and eruption of teeth of monkeys however. Edwards and Kitchen[4] found resection of sympathetic fibers caused 2-15% accelerated tooth growth in kittens, but resection of the inferior alveolar nerve on the other hand had no definite effect. Bishop and Dorman[5] found stimulation of the superior cervical ganglion caused vasoconstriction in the mandibular artery and severing the mandibular nerve resulted in increased blood flow in this artery.

Most of these authors have discussed the changes in the number and distribution of the nerves in the pulps of denervated teeth. Butcher and Taylor[3] found a decrease in the number of nerves although the pulps were otherwise normal. They concluded the maintenance of tooth structure did not depend on innervation. Christensen[6] found nearly all nerve fibers in the pulp disappeared following sectioning of the trigeminal nerve but removal of the superior cervical ganglion caused no appreciable change in amount or distribution of nervous tissue. Fernhead[7] sectioned the inferior dental nerve in the monkey and in one month only a few isolated nerves remained in the pulps of the teeth. The plexus of Raschkow had disappeared and no terminal fibers appeared in the predentin tubules, whereas in the control side intratubular nerves were found.

It is the purpose of the present investigation to utilize the method of resection of the nerves to mandibular teeth of rabbits to study the concentration and patterns of distribution of esterases in the pulps. Both gel electrophoresis and histochemical techniques are being carried out as well as silver staining of the pulp tissue to determine the success of the operations. In the future ultrastructural studies will be carried out as well.

METHODS

The inferior alveolar nerve and the superior cervical sympathetic ganglion were unilaterally resected from New Zealand white rabbits weighing between 2.5 and 4 kg. Before operating, the rabbits were sedated with chlorpromazine hydrochloride, the anesthesia used was sodium pentobarbitol (Nembutal) 20 mg/kg, and postoperatively cosa terramycin (Pfiser) was included in the drinking water

to prevent infection. The inferior alveolar nerve was approached from the medial aspect of the inferior border of the mandible. The nerve was sectioned as it entered the mandibular canal, and the proximal end sutured back into the internal pterygoid muscle to prevent any apposition of the cut ends. The superior cervical sympathetic ganglion is located lateral to the superior border of the larynx. It was removed by holding it in a hemostat and dissecting completely around it. A check on the success of the sympathectomy was done by comparing the size of the pupils of the eyes.

After 3-8 weeks, the rabbits were sacrificed with an overdose of Nembutal. Heparin was previously administered to reduce blood clotting. The animals' carotid arteries were then cannulated, the jugular veins severed, and the animal perfused with normal saline until the perfusate was clear and the ears and the oral mucosa became light pink or white. This procedure usually required about 2 liters of saline which was administered over a 45 minute period. After perfusion the mandible was dissected free and the inferior border was removed, exposing the apices of the tooth roots. Since the pulps are triangularly shaped with the base situated inferiorly they were easily removed by rimming the perimeter of the pulp chamber with a needle. One normal and one pulp from the denervated side of the same animal were placed in Bouin's PFA fixative; the others from each side were then pooled, frozen, and later prepared for electrophoresis. The pulps were homogenized in 0.5 M sucrose, centrifuged, and the supernatant electrophoresed according to the method described by Ornstein and Davis.[8] The gels were stained in 2.5×10^{-4} M α-naphthyl acetate and Blue RR diazonium salt.

To verify that the pulpal nerves on the operated side had degenerated, the pulps preserved in Bouin's PFA were sectioned and stained using a modification of Glees and Marsland's modification of Bielchowsky's silver stain.[9] The sections were incubated in the silver nitrate solution for only two minutes and then toned in gold chloride and later oxalic acid. The sections were later counterstained with Van Gieson's stain for collagen. This minimized the silver staining of the collagen and helped differentiate the collagen from the small, non-myelinated nerve fibers.

RESULTS

Although the results are incomplete at this time, the histological sections indicate that the inferior alveolar nerve and the superior cervical sympathetic ganglion had been severed, as the normal pulps showed numerous nerve fibers organized in the pulp in central trunks and peripheral plexus while those on the operated side had few nerve fibers that stained with the silver stain. It was also noted that the pulps on the operated side often demonstrated a change in the fibrous tissue. In these pulps the connective tissue fibers appeared larger, more dense, and heavily stained in comparison with the normal pulp tissue. By incubating in the silver nitrate solution a much shorter time

than recommended by Glees and Marsland,[9] the staining of the collagen fibers was reduced significantly while the nerve fibers remained quite dark.

The results of the electrophoresis are not complete but five different esterases have been separated, one of which is believed to be acetylcholinesterase. In the denervated teeth one band, which is located in the same region that cholinesterases is, is decreased in intensity in the denervated teeth, Fig. 1. The electrophoretic gels are now being scanned on a spectrophotometer and the optical densities of the bands are recorded in an IBM program that compares the relative densities of each band.

DISCUSSION AND FUTURE PLANS OF THIS PROJECT

It is desirable to determine differences in the esterase patterns by resecting one or the other of the two nerve supplies to the teeth. It is also of interest to find out more about the mechanism involved in the increased fibrosis in the pulps.

It is a constant aim to improve the localization and resolution of the electrophoretic patterns to facilitate a more accurate quantitative analysis of the esterases. Identification of the various electrophoretic bands will be done using selective inhibitors such as DFP and eserine sulfate. BW 62-C-47 diiodide and tetraisopropylphosphoramide (iso OMPA) can be used to further differentiate the cholinesterases. Electrophoresing nervous tissue or RBC's (both rich in AcChE) would help establish the location of the AcChE in the electrophoretic pattern. A method by Davis,[10] using dithiooxamide is reported to improve the resolution of the AcChE band, and will also be tried. Another electrophoretic technique that might be employed to improve the quantitation of the esterases is to electrophorese them off the end of the column and simultaneously elute them with a crossflow of buffer into a capillary tube and through a spectrophotometer.[11] The buffer can also contain α-naphthyl acetate and diazonium salt to stain the esterases in the capillary tube itself.

If the esterases can be selectively stained in the acrylamide gels, their localization in the tooth pulp will be attempted using the same staining procedures. At present, experiments are planned to utilize Karnovsky and Roots' modification of Koelle's thiocholine technique for the localization of AcChE and ChE.[12] These histochemical studies on both tooth slices and split teeth will be performed on denervated as well as normal tooth controls.

For more precise determination of AcChE activity, the entire tooth pulp homogenate (without centrifugation) can be incubated with acetylcholine in a pH-stat. By measuring the rate at which base must be added to the mixture to maintain a constant pH, the rate of hydrolysis of the substrate (and thus an indication of the AcChE activity) can be determined.

A comparison of the normal and denervated teeth will be considered ultrastructurally as well. Presence of nerves in dentinal tubules, possible changes in the odontoblast or its process or the cells in the pulp after denervation will be studied. The cell-rich zone will be studied as well as myelinated and non-myelinated nerve fibrils of the pulp.

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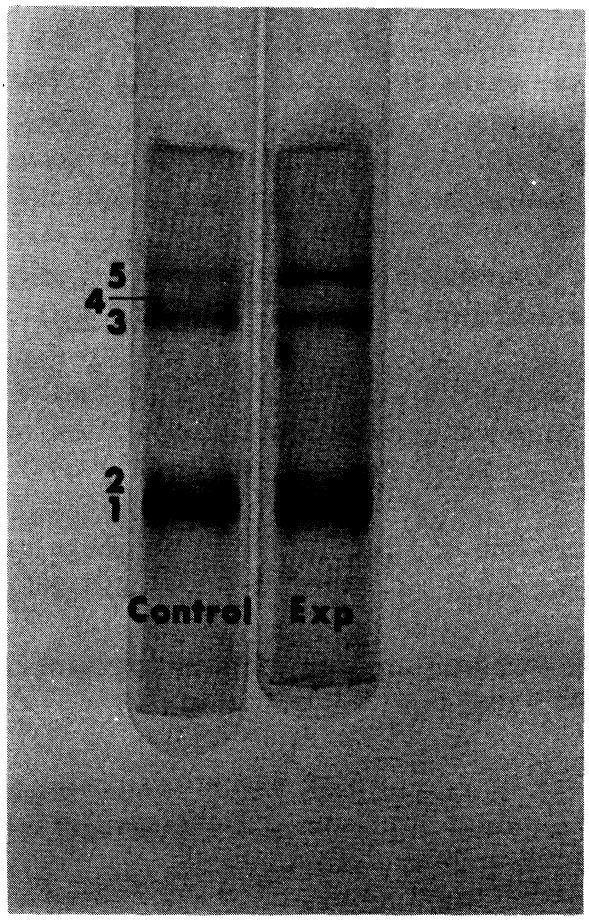

Normal rabbit tooth pulp (left) compared with denervated tooth pulp (right), as stained with α-naphthyl acetate and Blue RR diazonium salt. Note that five bands are seen in the esterase pattern of normal pulp tissue; while only four bands are present in the esterase pattern from the denervated side. The missing band is the one that appears fourth from the anode end (bottom) of the gel. This band appears very light in the control gel, and may be difficult to distinguish.

LITERATURE REVEIW AND RESULTS

There have been a few authors recently, such as Hattyasy,[1] Fernhead,[2] and Stella and Fuentes,[3] who claim light microscopic evidence of nerve fibers in the dentin. On the other hand, other investigators such as Zerosi,[4] Arwill,[5] Kerebel,[6] and Bernick,[7] found only incidental neural fibrils in the predentin zone and none in dentin. Electron microscope studies describing neural elements of the pulp have been carried out by Arwill,[5] Matthews, Dorman and Bishop,[8] Uchizono and Homma,[9] and Engstrom and Ohman.[10] Arwill[5] found two types of unmyelinated fibers in the pulp and in the dentin, he noted a few elements outside and at an angle to the dentinal tubules. Uchizono and Homma described the ultrastructure of both the myelinated and unmyelinated nerves of the pulp. Engstrom and Ohman[10] showed electron micrographic evidence of unmyelinated pulpal nerve fibers and Matthews, et al., noted the autonomic nerve fibers in contact with the smooth muscle cells of arteries. A search for neural elements in dentin has been carried out by Arwill,[5] and in 1966 by Frank.[11] Arwill[5] studied 2,300 electron micrographs and found no evidence of neural elements but Frank[11] illustrates what he believes to be unmyelinated neural elements in the dentinal tubules. These he describes as being 0.5 to 0.7 μ in diameter lying adjacent to the odontoblast process within the dentinal tubule. The axoplasm of these fibers contain numerous mitochondria and some synaptic vesicle-like structures. Although the fibrils are not particularly distinct, the structures described may not actually be nerve fibers. They appear as plasma membrane enclosed fibrils containing mitochondria and vesicles. They appear more like part of the odontoblast process and may be the side branches of this process. These have been described earlier and are visualized as running along the dentinal tubules for a distance and then passing abruptly at a right angle to an adjacent dentinal tubule by means of connecting canaliculi Avery and Rapp.[12] The electron micrographs shown in the present study (Fig. 1 and 2) do not reveal the presence of any such small fibers. These photographs are taken near the dento-enamel junction in young dentin and show the odontoblast process completely filling the tubule. The difference in findings could be due to the age and/or the location of the processes. It is possible that secondary processes or neural elements do not exist in young dentin of a newly forming tooth. It is thus one of the purposes of the present study to identify any possible neural elements or other structures in dentinal tubules and to determine when they arise, how long they exist, their possible functions and any relationships they may have with the odontoblast. The ultrastructure of the odontoblast and its process are also of great interest. In addition to studying the side branches of this process, its extensions into enamel (spindles) will also be studied. The cytoplasm of the cell will be studied to determine if synaptic organells are present. Interconnections between the odontoblasts

are well known and have been described by a number of electron microscopists, one of the first being Watson and Avery.[13] These intercellular attachment areas appear located at the pulp-predentin border in the area where the cell process enters the predentin-dentin tubule. Frank[11] believes the attachments between odontoblasts to be desmisome-like but what he shows do not appear as tight junctions as described by Farquhar and Palade.[14] In the present studies the authors have found nexuses-like structures located near the nuclei of the odontoblasts. These attachments are thus at the far end of the cell from the above mentioned intercellular attachments. Dewey,[15] defines nexuses to be zones of fusion of the plasma membranes of adjacent cells. Other authors report that such structures offer pathways of electric current between cell interiors.[16-19] It may be possible that they constitute a structural basis for electrical transmission in these systems. The cell will be studied relative to adjacent neural fibers and the cell-free and cell-rich zones. The latter zone will be carefully examined as it is claimed by Kubota and Kubota[20] to be composed of such nervous tissue elements as Schwann cells and endo- and perineural connective tissue cells. They believe that latter have been discarded during the course of plexus formation and is a zone of demyelination of nerve fibers along the front of dentin formation. Because of the voluminous nature of light microscopic description and discussion about this zone its ultrastructural characteristics will be determined. All of these studies will be carried out on the developing, maturing, exfoliating and aging pulps of man as well as in denervated teeth and in teeth affected by carefully controlled dental operative cutting procedures.

METHODS

Although it is anticipated that as many changes in the future will be made as in the recent past, the following general techniques are now in use. Tissues will be fixed in 2% glutaraldehyde in a phosphate buffer at 4°C for 30 minutes to two hours. Teeth needing decalcification will be placed in small gauze bags and suspended in 250 cc's of 3.2% E.D.T.A. decalcifying solution at 4°C. The solution will be periodically changed. The length of the decalcification period depends on the size of the calcified mass a predetermined by a decal schedule. The larger teeth will be cut into blocks 1 mm^3 and washed in phosphate buffer and postosmicated in 1% osmic acid at 4°C for 2 hours, dehydrated through a series of graded ethanol, held in two separate changes of propylene oxide and embedded in Epon 812. The decalcified blocks will be sectioned with glass knives with an LKB ultramicrotome. A number of undecalcified teeth will be sectioned with the use of a diamond knife. Sections will be stained with uranyl acetate and some will be stained with a combination of both uranyl acetate and lead hydroxide. Permanent fixation may be required for the study of the membranes of the odontoblasts, (especially areas of nexuses). An R.C.A., EMU 3D electron microscope will be used for observation and photography of the sections.

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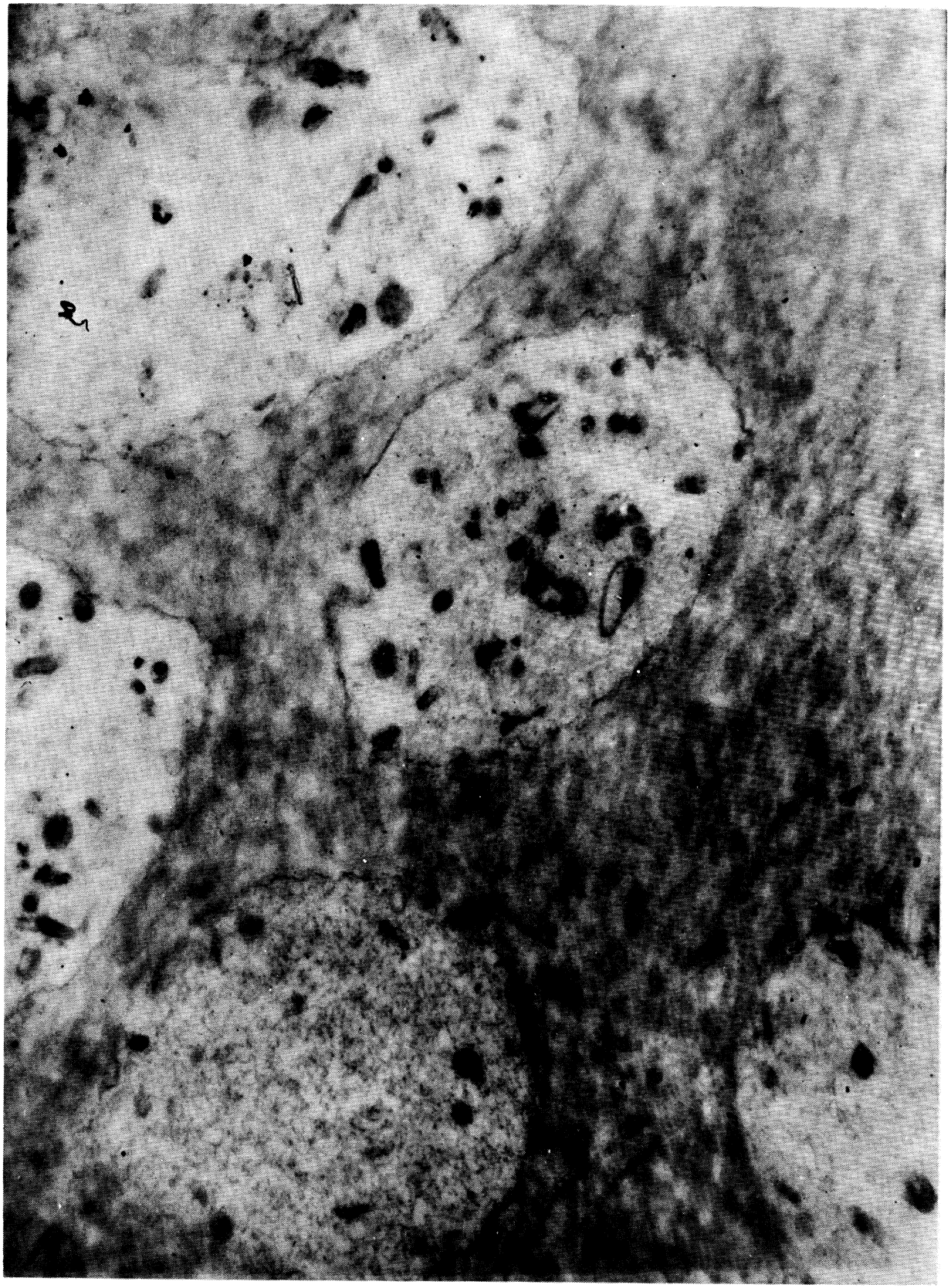

Fig. 1. An electron micrograph of a transverse section through odontoblastic processes within dentin tubules. Observe the random oriented collagenase matrix of the dentin surrounding the tubules. The cytoplasm of the process contains many small filaments and a few larger irregular shaped bodies.

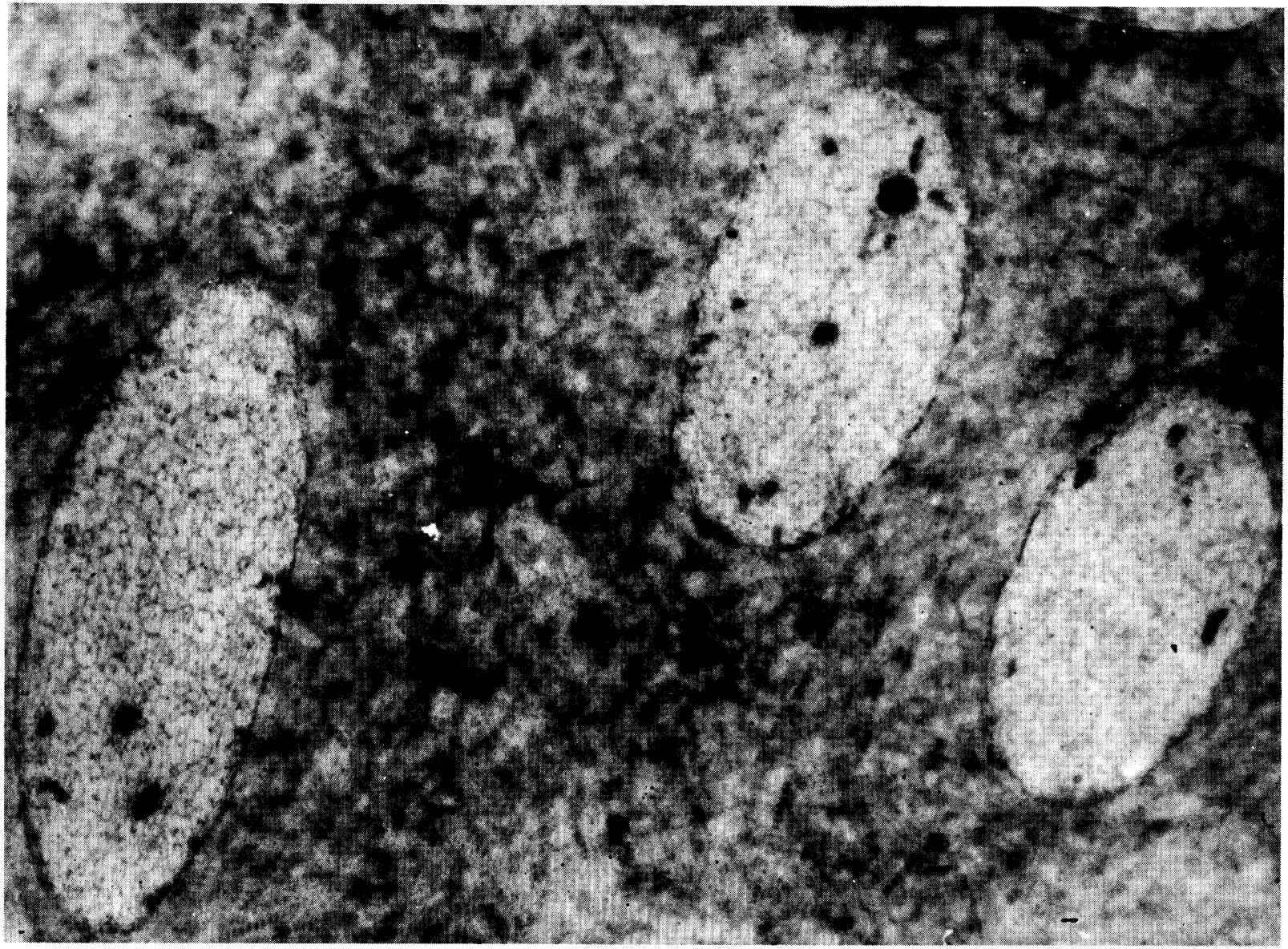

Fig. 2. An electron micrograph of a transverse section of odontoblastic processes. The smaller filaments appear to form a cytoskelton in the process. The processes appear to entirely fill the tubules. The typical 640 Å banding of the collagen of the dentin matrix is observed.

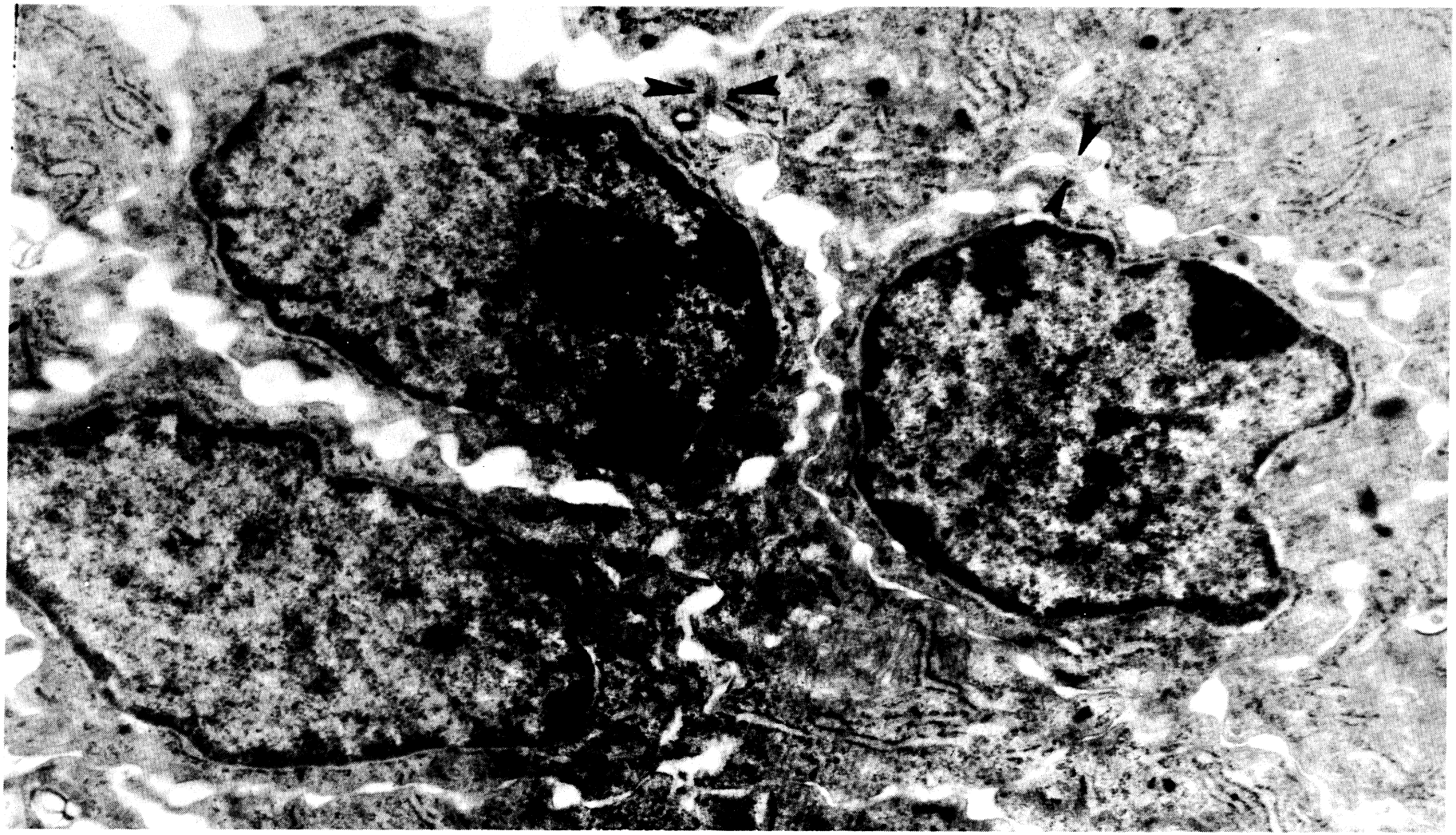

Fig. 3. An electron micrograph of a transverse section through the odontoblasts. The gulteraldehyde fixed nuclei show the characteristic clumping of the ribosomal material near the periphery of the nuclei. The cells show abundant rough surface endoplasmic reticulum, characteristic of the area near the odontoblastic nuclei. Although there is some separation between cells, zones of possible nexus or zona occludens (tight junctions) are seen, arrows.

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4. FOREIGN TRAVEL

None

5. OTHER CONTRIBUTIONS

Interest in the pulp and the mechanism of neural conduction by the principal investigators has carried over into the undergraduate and graduate programs. Also the investigators were active in the organization of a conference and presentation of research data at that conference on the pulp.