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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6. Summary Statement:

   This project began June 1, 1967, and proposed an ultrastructural and histochemical investigation of the contents of the dentinal tubules from the dento-enamel junction to the pulp and to relate these findings to the neural conducting system of the pulp. It was proposed that this investigation be carried out on developing teeth to determine when and to what extent that neural elements enter the pulp and whether they are incorporated in the forming dentin. It was also proposed to study the effects of denervation on the contents of the dentinal tubules, the odontoblasts and the pulp organ. Therefore, resection of either the inferior alveolar or cervical sympathetic nerves or both of New Zealand rabbits was carried out and the results compared to nonresected teeth. Using this experimental approach, gel electrophoresis and histochemistry of the cholinesterases and ultrastructural studies of the teeth have been carried out. As this study progressed, titrometric methods were used for cholinesterase

determinations as this was found to be more accurate and quantitative than the gel electrophoretic methods. These studies revealed a significant decrease in the concentrations of cholinesterase in the incisor of pulp organs 15-19 days after resection of either the inferior alveolar (I.A.N.) or both the I.A.N. and superior cervical ganglion (S.C.G.). Only a slight decrease in concentration was noted after removal of the superior cervical ganglion alone. Histochemical results revealed a decrease in cholinesterase after resection of either or both I.A.N. or S.C.G. The reverse seemed true of the concentration of the oxidative enzyme succinic dehydrogenase. Nerve stains confirmed the loss of nerve trunks after I.A.N. resection and the loss of blood vessel associated nerves after S.C.G.—resection and both after both resections. Dilatation of the blood vessels was prominent after S.C.G. resection.

During this past year ultrastructural studies of the contents of the developing dentinal tubules revealed the process of the odontoblasts to contain numerous organelles which decrease in number as dentin formation proceeds. The minute side branches of these processes were also observed. These latter secondary processes of the odontoblasts recently may have been mistaken for neural elements by one investigator. Finally, a system of tight junctions have been found to exist between the odontoblasts which may offer pathways of movement of ions between cell interiors. It is proposed now to study this system ultrastructurally in the I.A.N., S.C.G. and combination, denervated teeth. It is further proposed to study the electrical resistance between these cells after various types of stimulation. Further studies on the effects of changes in the intrapulpal pressure and the role of the polypeptide kinins in the teeth is also proposed. The response to cavity preparation and to various types of irritants will be evaluated on the resected and nonresected model. It is hoped that this multidiscipline approach will bring us closer to the understanding of these complex phenomena of neural transmission in teeth. Past and recent studies on the histology, physiology and biochemistry of the teeth make it profoundly clear that there is no simple explanation to this mechanism.

B. DETAILED REPORT

1. DESCRIPTION OF RESEARCH

Alteration of Teeth After Nerve Resection

INTRODUCTION

The purpose of this investigation is to study the changes in the pulps and dentin of teeth after resection of their nerve supply. It is hoped that by studying the effects of the absence of these structures in the pulp organ, a clearer concept of their total function may be gained. For this purpose in one series the inferior alveolar nerve (I.A.N.) was resected, in the second the cervical sympathetic (S.C.G.), in the third both were removed and in the fourth neither. In addition to comparing the distribution of cholinesterase and the oxidative enzyme succinic dehydrogenase by histochemical means, histologic study of the pulp tissue and dentin was planned as well as quantative determinations of the cholinesterase levels by titrimetric means. Several investigators have reported the effects of denervation on the growth patterns of teeth. King[1] sectioned the cervical sympathetics in rabbits and found a temporary acceleration of growth of the incisors which he attributed to the vado-dilatation which resulted. Taylor and Butcher [2,3] found a 20% to 30% increase in eruption of rat incisors after inferior alveolar nerve resection. They concluded this was due to the lack of sensation which caused a tooth fracture resulting in the lack of occlusion which in turn allowed an increased eruption rate. Edwards and Kitchen[4] noted resection of sympathetic nerves 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 of 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 decreased number of nerves, although the pulps appeared otherwise normal. They concluded that 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 fifth nerve. Removal of the superior cervical ganglion caused no appreciable change in distribution. Fernhead[7] sectioned the inferior alveolar nerve in the monkey and after one month only a few isolated nerves remained in the pulps of teeth. Weatherred[12] stimulated the peripheral cut end of the inferior alveolar nerve and caused an increase in the ultrapulpal pressure measured with a sensitive pressure transducer in contact with the pulp. Stimulation of the

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cervical sympathetic ganglia on the other hand produced a decrease in pulp pressure collection of samples of saline from a cavity in contact with the pulp normally contained little smooth muscle stimulating substance until stimulation of the alveolar nerve. The contractions resembled those obtained with synthetic bradykinin. Kroeger and Krivoy[11] found the rat incisor to contain enzymatic activity capable of destroying the smooth muscle stimulating substance of both the active material of the tooth pulp as well as synthetic bradykinin.

METHODS AND MATERIALS

New Zealand rabbits were selected for this study as they have continually erupting incisor teeth which provide an ideal means of study of metabolically active pulps. In this manner absence of innervation can be studied on the formative areas as well as the older zones of the pulps. A total of 30 animals weighing between 2.0 and 4.0 kg were utilized. The rabbits were initially sedated with 1.5 cc of a 1:1 sodium pentobarbitol 50 mg/cc and a 0.9% sterile sodium chloride, administered in the marginal vein of the ear. An intraperitoneal route was then maintained during the operation to provide anesthesia. Resection of the superior cervical ganglion or inferior alveolar nerve or a combination of both were then carried out. After surgery the time allowed for degeneration of the nerves was from 15 to 19 days. Each operation was done on the right side, while the left remained unoperated and served as a control. Terramycin was included in the drinking water post-operatively to prevent infections.

The right inferior alveolar nerve was approached from the medial aspect of the inferior border of the mandible (Fig. 1). The periosteal attachment of the medial pterygoid muscle to the mandible was separated in approaching the mandibular foramen. It is located just medial and approximately 1-1/2 cm above the point where the facial artery crosses the inferior aspect of the mandible. Surgical silk was tied around the nerve approximately 5 mm from the foramen and the nerve was sectioned distal to this point. Visual observation of the remaining distal stump was then made and the proximal end was sutured to the medial pterygoid muscle to prevent reapposition. At the time of sacrifice a second dissection of the area was made to determine whether the inferior alveolar nerve had remained ligated. The right superior cervical ganglion was located by blunt dissection of the omohyoid and sternohyoid muscles to expose the carotid sheath adjacent to the trachea. Longitudinal dissection exposes the sympathetic trunk and the superior cervical ganglion was located just lateral to the larynx. In all cases the right superior cervical ganglion, with its cranial branches was removed along with a portion of the ascending sympathetic trunk. Success of the operation was noted by comparing the pupil size of the right to the left, a decrease of the right pupil was evident. After surgery the time allotted for degeneration of the nerves was from 15 to 19 days. At the end of this time the animals are sacrificed, the entire mandible removed and both the left and right sides are run concurrently.

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Other studies are planned and in progress using the resected tooth model. For example, cavity preparations are prepared in these teeth and the rate and type of formation of reparative dentin will be studied. Studies using irritants will also be tried in an effort to learn more about the mechanism controlling pulpodentinal response. All tissues resulting from the experimental procedures will be prepared by the following techniques.

Tissues were prepared for histologic observation by fixation in 10% neutral buffered formalin at 4°C. Decalcification with formic acid buffered with sodium citrate and embedded in Paraplast and serial sectioned from 10-16 microns. Staining procedures used were either a standard H. & E., Mallory or Masson techniques or the Lison method using an alcian blue 8G with chlorantine red fast 5B. The alcian blue selectively stains structures containing mucopolysaccharides. Rowle's silver stain a modified Ungwitter stain, has been found successful for controlled impregnation of nerve fibers, using Sym-Collidine buffer to maintain a pH at 7.3. Holme's silver stain, employing a Van Gieson's counter stain is being used as well.

Histochemical procedures were employed for both normal and resected nerve pulps. Tissues were removed immediately after sacrifice of the animal and frozen in CO_2. Sections were cut on a cryostat and mounted serially and the localization of cholinesterases was studied using both the Koelle[13] and the Karnovsky-Roots[14] technique. The latter direct coloring technique was utilized with a 10^{-6} M solution of DFP to inhibit the nonspecific cholinesterases. The tissues were also analyzed for the respiratory enzymes succinic and lactic dehydrogenases, and glucose 6-phosphate using nitro BT, which results in a blue diformazan precipitate, at the sites of the activity.

Although the gel electrophoresis technique was useful in identification of isozymic forms of cholinesterases present, the technique did not allow sufficient quantitation. Therefore, titrimetric methods as described by Augustinsson[15] were used to analyze the cholinesterase (AChE) activity. The entire pulps were used and incubated with acteycholine substrate which resulted in a release of choline and acetic acid. The latter was titrated with NaOH. The rate of addition of base sufficient to maintain a constant pH, reveals the degree of hydrolysis of the substrate and activity of the AChE enzyme. Upon sacrifice of the animals the pulps were removed, weighed, and homogenized at 4°C, and mixed with 1 cc of distilled water for each 50 mg of pulp. Titrations were carried out on a pH meter calibrated in 0.01 pH units. The reaction mixture was made of equal volumes of 1.5 cc of pulp preparation and 1.5 cc of 6×10^{-3} M acetylcholine chloride which was placed in a 5 cc beaker and mixed with a magnetic stirring rod. The pH of the mixture was usually brought to 8.0 in less than 4 minutes. After this period readings of the micrometer dial on the microsyringe were recorded at 1 minute intervals. The latter was expressed as millimicromoles of NaOH added per time elapsed.

For each type of operation, (1) I.A.N., (2) S.C.G., (3) and combination, the rates of reaction were calibrated individually as well as in groups of four

control and experimental. The rate of reaction in this study, is an expression of millimicromoles of 10^{-4} NaOH that is needed to maintain a constant pH of 8.0 resulting from hydrolysis of the substrate (acetylcholine). In each case the unoperated side served as the control and was titrated under the same conditions as the operated side. For each rabbit representing a control and experimental run, a fresh solution of acetylcholine chloride (Merck) 3×10^{-3} M was prepared to maintain a constant working substrate solution. The pH meter was recalibrated before each run to 0.01 of a pH unit using a pH 7.0 buffer solution, and the temperature was kept constant at 25°C for the duration of the run.

RESULTS

A. Histologic

Fifteen to nineteen days after resection of the inferior alveolar nerves of the rabbit incisor all main nerve trunks as well as the fine terminations throughout the pulp were missing. In a few cases signs of a degenerating segment of nerve trunks could be found with the Rowle's nerve stain. The absence of nerves contrasted sharply with that of the adjacent control pulp. Nerves were visible along the blood vessels, however the pulp appeared otherwise similar to the control (see Figs. 3,4,7, and 8).

Resection of the superior cervical ganglion had a dramatic effect on the pulp. All of the blood vessels appeared dilated. The dilated capillaries were apparent even among the odontoblasts. Neural stains failed to reveal any nerves in the walls of the blood vessels although larger neural trunks were seen throughout the pulp. None of the latter were associated with blood vessels, however. The dilation of the pulp vessels appeared to crowd the substance of the pulp to some degree. Otherwise, this connective tissue appeared like the normal (see Figs. 5 and 9).

Removal of both the inferior alveolar and superior cervical nerves caused the most noticeable effect on the pulp. The blood vessels of the pulp were dilated throughout and silver stains revealed no evidence of any neural elements either in the walls of the vessels or elsewhere (see Figs. 6 and 10).

Of of the more interesting aspects of the resection studies can be seen in Figs. 23-26. In the I.A.N. resected teeth there is a small opening in the dentin at the tip of the incisor. The lack of dentin formation has resulted in a small tubular defect which is filled with necrotic cells. This tubule appears open all the way to the tip of the tooth. In the S.C.G. resected teeth the open tubule is more apparent being larger and filled with blood vessels and living cells. Near the outer tip of the incisor the cells appear necrotic. In the combination I.A.N., S.C.G. the condition is even more exaggerated. The

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dentin appears gnarled or irregular information with a defect opening to the outside. Small vessels and cells fill this defect except near the surface where a plug of dead cells exist.

B. Histochemical

Resection of the inferior alveolar nerve resulted in a reduction of specific cholinesterase in the dental pulp. This is due to the absence and thus lack of staining of pulp nerves. The amount of ChE in the blood vessels appeared the same as in the nonresected pulp (Figs. 11 and 12). A definite reduction of ChE in the odontoblast was observed as compared to the control (Figs. 15 and 16). On the other hand there appeared to be heavier staining of succinic dehydrogenase than in the control (Figs. 19 and 20).

Removal of the superior cervical ganglion resulted in increased cholinesterase in the pulp due to the dilated blood vessels but a conspicious absence in neural trunks (Fig. 13). Similar to the I.A.N., the S.C.G. revealed less ChE in the odontoblast and more succinic dehydrogenase (Figs. 17 and 21).

When both the S.C.G. and I.A.N. are removed the dilated vessels again give the pulp the appearance of an increased amount of cholinesterase concentration. The absence of nerves, however, shows a conspicious absence of the dark cholinesterase stained nerves in the pulp (Fig. 14). Again, the odontoblast has little or no cholinesterase as compared to the control, but an increase in the succinic dehydrogenase (Figs. 18 and 22).

C. Titrimetric

The inferior alveolar nerve resection (I.A.N.) series revealed higher individual rates of reaction for the control side opposed to the experimental side. Figure 27 demonstrates the rate of reaction for the control side as 3.100×10^{-5} M or 11.60 mμ moles of base utilized during the titration procedure as opposed to 2.103×10^{-5} M or 6.10 mμ moles of base for the experimental I.A.N. side. This represents an individual reduction of 5.50 mμ moles of base on the operated side. A comparison of the entire I.A.N. group and their control counterparts revealed a combined rate of reaction of 4.89525×10^{-5} M for the controls against a 3.5942×10^{-5} M reaction rate for the I.A.N.'s. This difference is represented as a significant decrease of AChE activity resulting from the resection of the I.A.N.

In the series run on the S.C.G. operations, there were individually observed higher rates of reaction for control pulps compared to the experimental S.C.G. pulps. Figure 28 shows one example from the four observations regarding this S.C.G. series. The rate of reaction for this particular run demonstrated a 4.068×10^{-5} M or 11.80 mμ moles of base utilized, while the operated

S.C.G. pulp exhibited a 3.655×10^{-5} M or 10.60 mμ moles of base titrated for the duration of the run. The difference of 1.20 mμ moles is explained as resulting from the resection of the S.C.G. The combined S.C.G. runs exhibited combined rates of reaction of 4.4153×10^{-5} M for the control group as against a combined reaction rate of 3.9310×10^{-5} M for the S.C.G.'s. This difference is hardly significant.

The series involving the I.A.N.-S.C.G. operations also revealed a decrease of AChE activity resulting from resection of both nerves. Figure 29 shows a comparison of the rates of reaction of the control side as well as the operated side. The control pulp demonstrated a rate of reaction of 4.444×10^{-5} M or $14.$—mμ moles of base while its operated counterpart revealed a rate of reaction 3.379×10^{-5} M or 9.30 mμ moles of titrated base. The difference of 4.20 mμ moles of base represents the change of acetylcholine hydrolysis resulting from resection of both I.A.N. and S.C.G. A comparison of the average rate of reaction of the control group for this series is 4.6022×10^{-5}, as against a 3.758×10^{-5} reaction rate for the I.A.N. and S.C.G. group.

A review of the total number of controls from the three groups reveals an average rate of reaction of 4.6369×10^{-5} with a deviation of not more than 0.2583×10^{-5} M at any extreme. The volume of base titrated for the entire control group was fairly constant at 12.46 mμ moles. It can be observed that the greatest reduction of base was observed in the resection of the I A.N. with the S.C.G. resection demonstrating the least change of total mμ moles of base needed to titrate against the hydrolysate.

In order to demonstrate the significance of each series, the degree of probability for each group were statistically computed. Figure 30 shows the acelycholinesterase activity that was observed from each pulp homogenate representing the mμ moles of base that was titrated each minute against the total volume (mg) of tissue. As seen in this figure, the most dramatic alteration of AChE activity occurs as a result of resectioning the inferior alveolar nerve while little change was found after sectioning the superior cervical ganglion.

Resection of both I A.N. and S.C.G. results in a significant reduction of the mμ moles of base that may be titrated against the hydrolysis of the substrate.

DISCUSSION

It is believed that the constantly growing incisor of the rabbit is a good experimental model for studying the effect of loss of the I A.N. and S.C.G. nerve supply. It is possible that at the base a zone of cell proliferation is evident while at the tip of the tooth the cells are undergoing degeneration. It is probable that the physiologic function of the odontoblast,

for example, will vary with its location in the pulp. In 15 to 19 days it is possible to see the effects of removal of either the I.A.N. or S.C.G. nerve supply. Rowle's stain has been noted to be particularly good in demonstrating the nerves in the pulp tissue. The effect of resection of the S.C.G. is more dramatic because of the dilatation of the vessels throughout the pulp. Alteration in dentin formation after resection agrees with the findings of Weatherred.[12] He noted imparement of dentin formation in the rat after I.A.N. resection. He noted these changes appeared when the post-denervation dentin became subject to wear. He found no changes in the dentin of the molar teeth which agrees with our observation in the rabbit. It is probable that intra-pulpal pressure increases as a result of vascular dilatation. Defective dentin occurs in both the S.C.G. and I.A.N. affected animals. It was noted that there was no evidence of vascular dilation appeared in the I.A.N resected teeth. It is possible that the nerves play some role in the incremental pattern of dentin formation and this will be an area of future interest.

The significant drop in cholinesterase after resecting of the I.A.N. provides support that acetylcholine is functioning in this neural system. Although there has been past and present evidence of the presence of AChE in the pulpal nerves demonstrated by histochemical methods the titrimetric techniques present a clear picture of the differences in quantity. It is of interest that resection of the superior cervical ganglion effects only to a slight extent the ChE concentration. This may in fact be due to the amount of ChE in the blood. The nonresected odontoblast of the rabbit pulp contains some cholinesterase in it which decreases to a great extent when the I.A.N. is resected. This is also true when the S.C.G. or both are resected. In both cases the concentration of succinic dehydrogenase increases after resection.

These observations illustrate the need for a future combined electro-physiological, biochemical, and ultrastructural approach to the study of the neural conducting system in denervated as compared to normal teeth. Further studies on the interrelationship of changes in the intrapulpal pressure and the concentrations of the polypeptide kinins in the teeth is also proposed. The response to cavity preparation and other irritants will be evaluated on the resected and nonresected teeth. It is hoped that this multidiscipline approach will bring us closer to the understanding of these complex phenomena. Past and recent studies on the histology, physiology and biochemistry of the teeth make it profoundly clear that there is no simple explanation of this mechanism.

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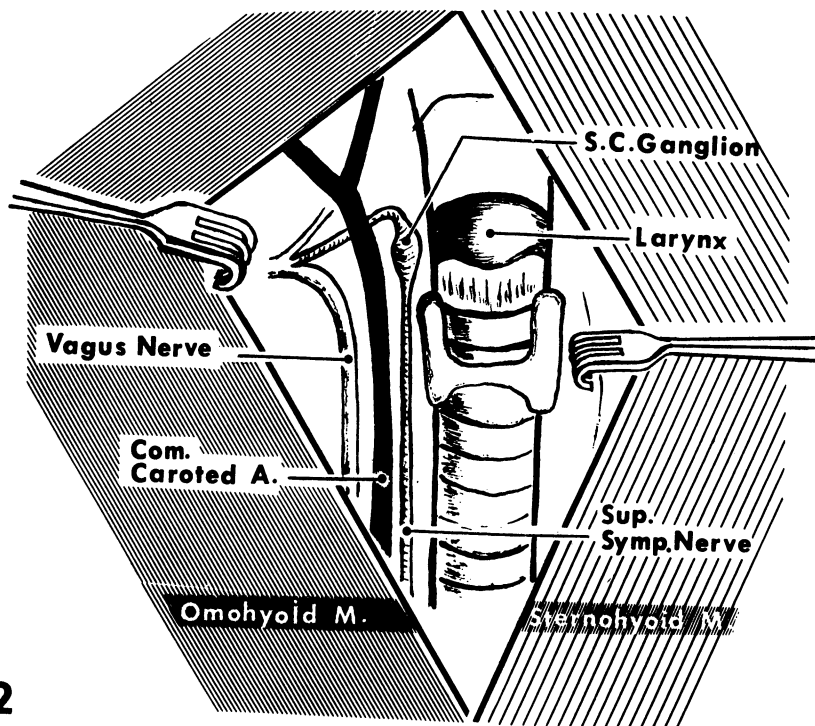   _chemical_ _Analysis_., Vol. V: Chap. 1., 1957.
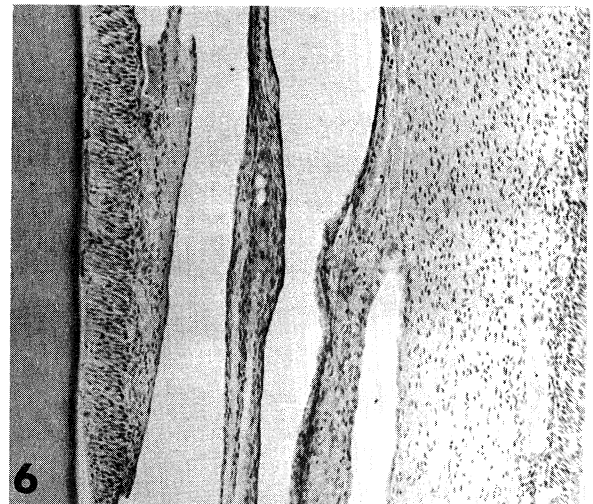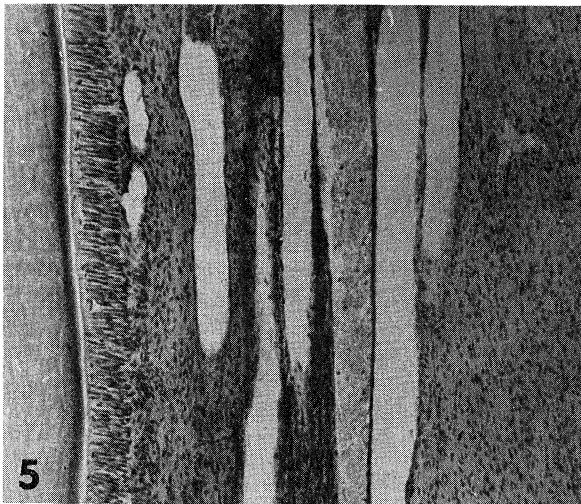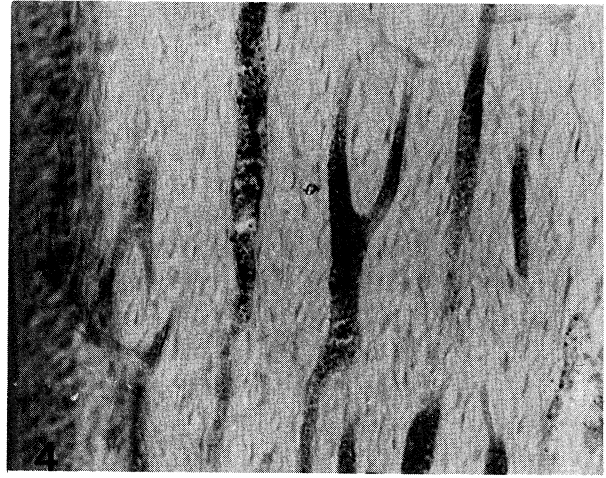
EXPLANATION OF FIGURES

Fig. 1   The approach to locate, tie and section the inferior alveolar nerve
is shown. The periosteal attachment of the medial pterygoid muscle
was removed from the angle of the mandible. The nerve was dissected
proximally for a few mm. A silk suture was tied around the nerve
and it was then resected distal to the suture. The nerve was then
sutured into the medial pterygoid muscle to prevent reapposition and
possible regeneration of the I.A.N.

Fig. 2   A schematic approach for the removal of the superior cervical gan-
glion. The strap muscles (not shown) were dissected away to reveal
the carotid sheath, whereby location and further dissection of the
sympathetic trunk was completed cranially. The entire superior cer-
vical ganglion was removed along with its cranial branches and sev-
eral mm of the sympathetic trunk.

Mandible
Inf. Alv. Folamen
Facial A.
Periosteum
Inf. Alv. Nerve
Med. Pterigoid M.

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S.C. Ganglion
Larynx
Vagus Nerve
Com.
Caroted A.
Sup.
Symp. Nerve
Omohyoid M.
Sternohyoid M.
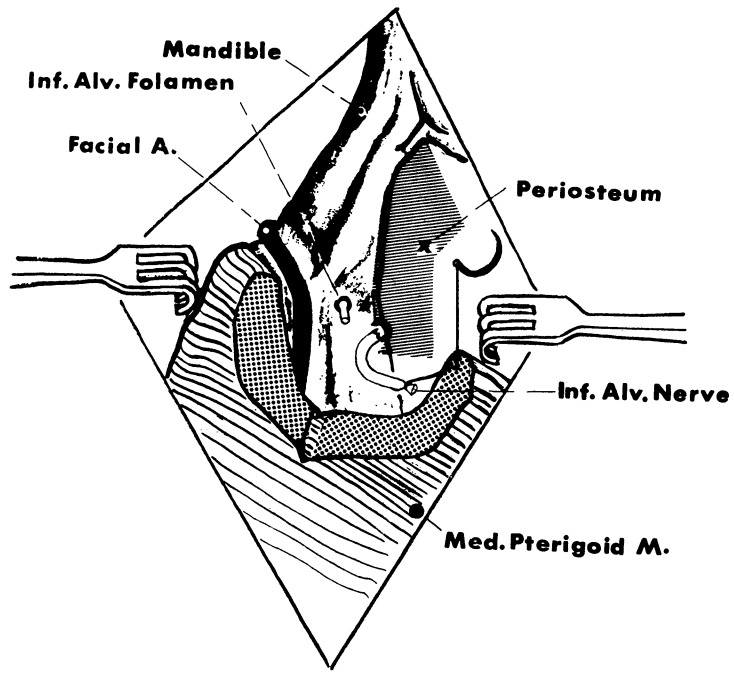
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EXPLANATION OF FIGURES

Fig. 3   A longitudinal section through the middle third of a normal rabbit
         incisor. The vessels are normally dispersed with the pulpal cells
         also distributed evenly. The odontoblast zone is shown, a cell rich
         or cell free zone is not evident. Masson trichrome X 25.

Fig. 4   A longitudinal section of an I.A.N. rabbit incisor (19 days). The
         vessels are normally distributed without any observable dilatation.
         There is no observable alteration of the odontoblasts or the pulpal
         cells. Masson trichrome X 60.

Fig. 5   A longitudinal section through the middle third of a S.C.G. (19 day)
         rabbit pulp incisor. There is noted dilatation of the larger vessels
         as well as those smaller vessels paralleling close to the odontoblasts.
         There is little change of pulpal cell dispersement or of the gross
         morphology of the odontoblasts. Masson trichrome X 50.

Fig. 6   A longitudinal section through the middle third of a (19 day) I.A.N.-
         S.C.G. rabbit incisor pulp demonstrated dilatation of vessels. There
         is no alteration of the odontoblast morphology nor pulp cellular dis-
   persement. Masson trichrome X 50.
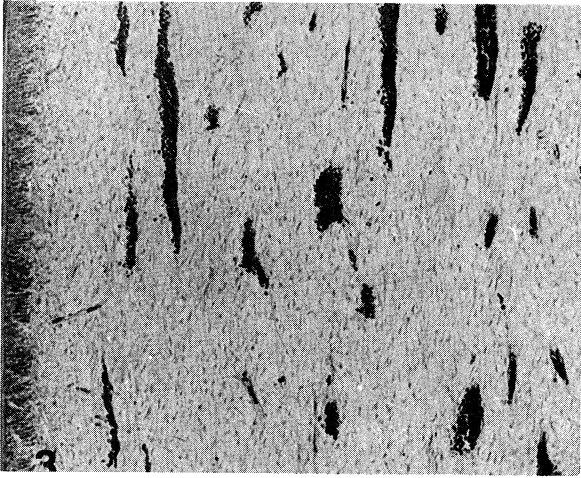
EXPLANATION OF FIGURES

Fig. 7   A longitudinal section through a control rabbit incisor exhibiting
         nerve trunks within the pulp (myelinated) as well as finer nerves
         (unmyelinated) running along, and on the vessels. Rowle's silver
         stain. X 70.

Fig. 8   A longitudinal section through an I.A.N. rabbit incisor (19 days).
         There is loss of all large nerve trunks as seen in the normal, how-
         ever, there still remain finer nerves directly on the vessels.
         Rowle's silver stain. X 70.

Fig. 9   A longitudinal section through an S.C.G. rabbit incisor pulp (19
         days). There is a dramatic reduction of fine (unmyelinated) nerves
         to be found along the vessels which are dilated. There may be seen
         several myelinated nerves throughout the pulp as well as a rather
         large nerve trunk. Rowle's silver stain. X 60.

Fig. 10   A longitudinal section through a I.A.N.-S.C.G. rabbit incisor (19
         days). There is complete loss of all large myelinated nerve trunks
         as well as the smaller (unmyelinated) nerves that are normally asso-
         ciated with the vessels. There is dilatation of the vessels as well
   as an observable alteration in the newly formed dentin. X 50.
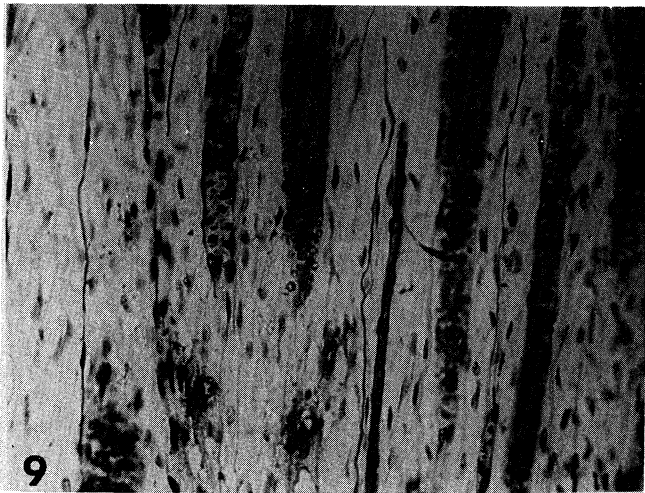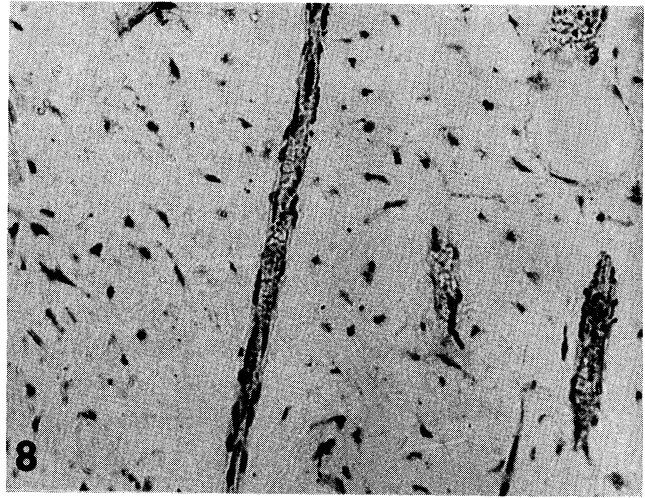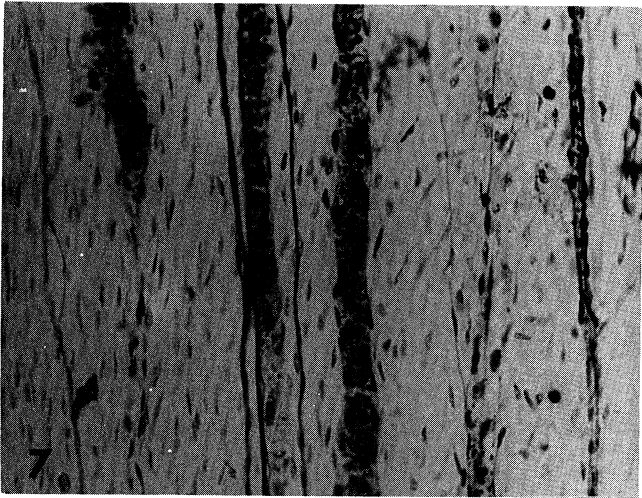
EXPLANATION OF FIGURES

Fig. 11   A section through the middle third of a control rabbit incisal pulp
          illustrating AChE activity along both nerves as well as the vessel
          walls. AChE & D.F.P. X 50.

Fig. 12   A section through the middle third of an I.A.N. rabbit incisor pulp
          (19 days). There is loss of activity along nerves however, it re-
          mains along the vessels. Observe areas of localizations along the
          vessel walls. AChE & D.F.P. X 60.

Fig. 13   A section through the middle third of S.C.G. rabbit incisor pulp
          (19 days) demonstrates a pronounced activity about the vessels as
          well as along the nerves of the pulp. Again observe the dense lo-
          calizations about the vessel walls. AChE & D.F.P. X 60.

Fig. 14   Represents a longitudinal section through I.A.N. & S.C.G. rabbit
          incisor pulp. There is loss of activity along the nerve trunks.
          The demonstration of cholinesterase remains along the vessel walls,
          retaining dense localization of activity. AChE & D.F.P. X 60.
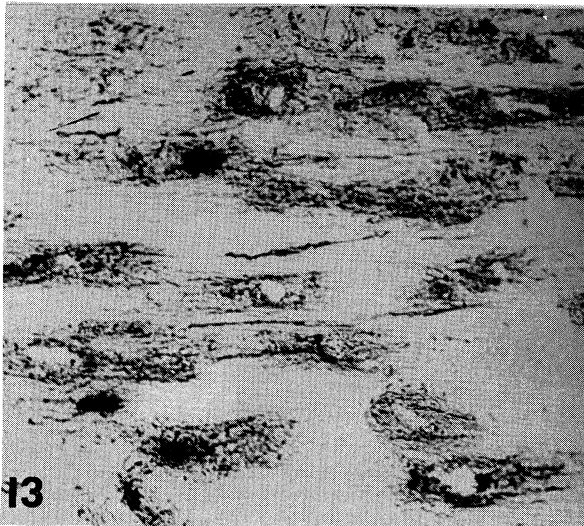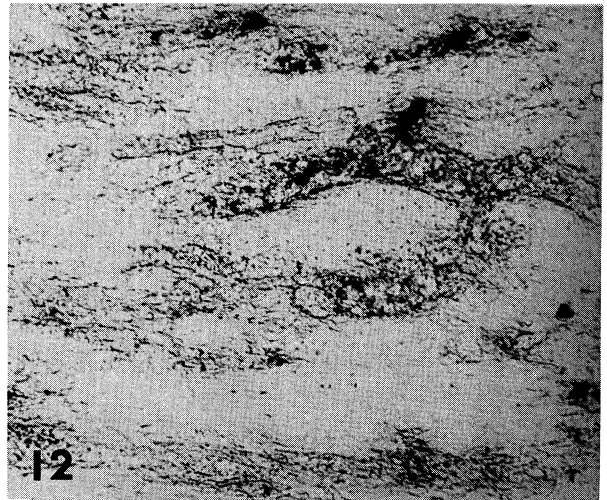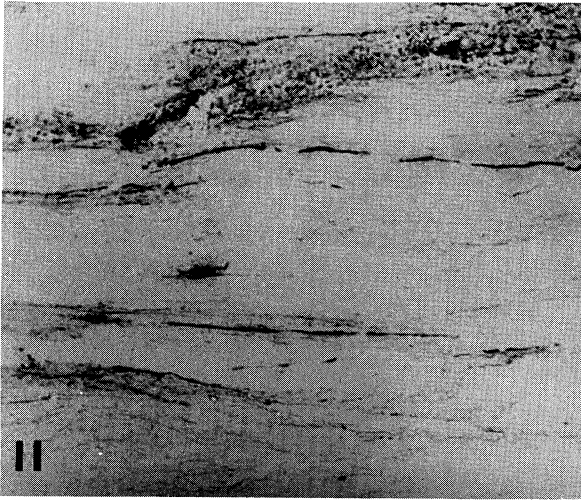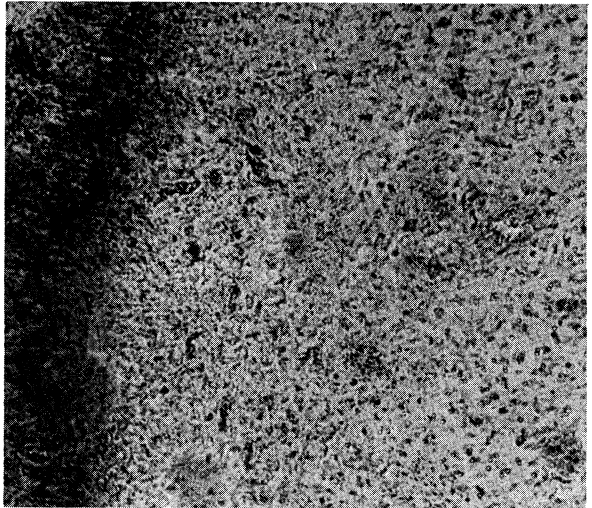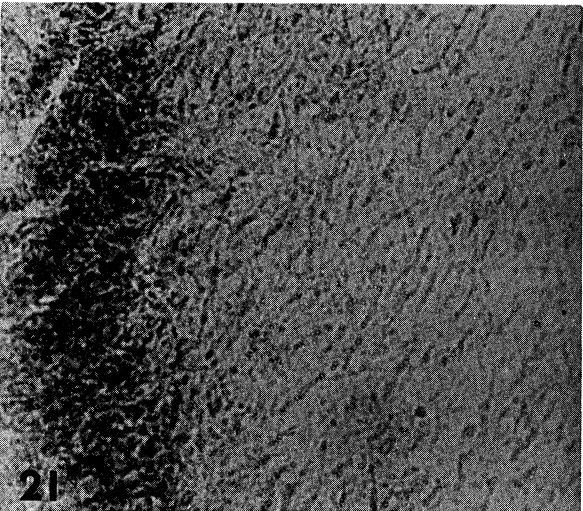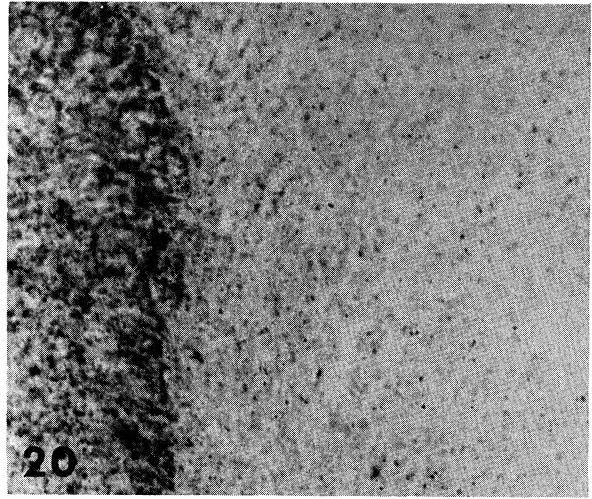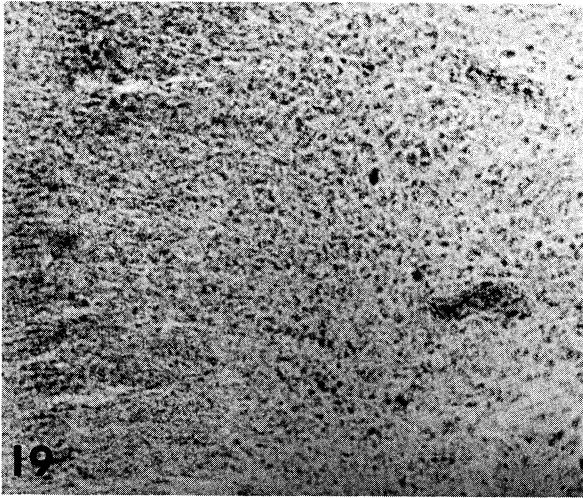
EXPLANATION OF FIGURES

Fig. 15   A section through a control rabbit incisor. There is definate cholin-
          esterase activity observed along the odontoblastic zone. Localiza-
          tion within the odontoblast appears to be indefinate and rather evenly
          dispersed. AChE & D.F.P. 40 microns. X 200.

Fig. 16   Represents a I.A.N. rabbit incisor (19 days) with an obvious de-
          crease in activity about the odontoblasts. AChE & D.F.P. 40 microns.
          X 200.

Fig. 17   A section through an S.C.G. rabbit incisor (19 days) demonstrating
          a reduction of cholinesterase activity in the odontoblasts. AChE &
          D.F.P. 40 microns. X 200.

Fig. 18   A section through the odontoblast zone of an I.A.N. & S.C.G. (19
          day) rabbit incisor. There is a rather dramatic reduction of cholin-
          esterase activity that may be observed. AChE & D.F.P. 40 microns.
          X 200.

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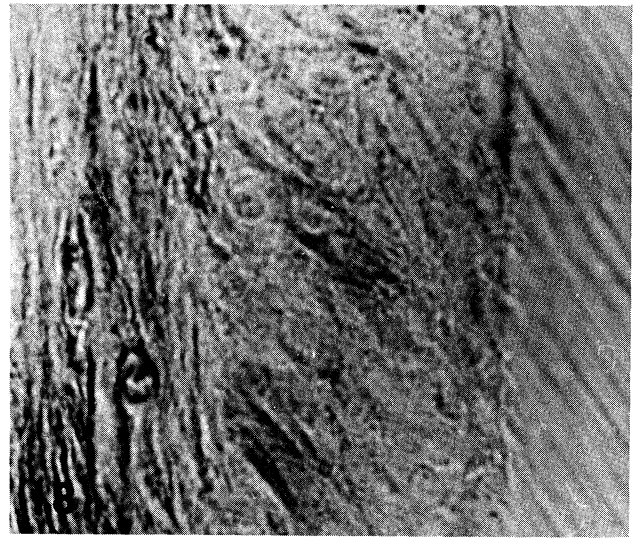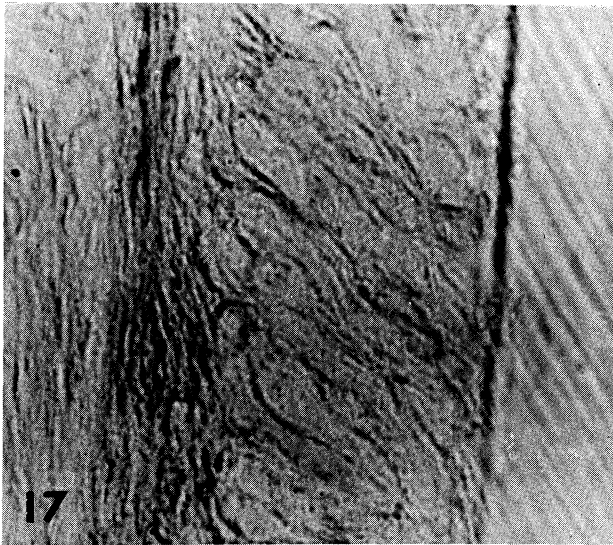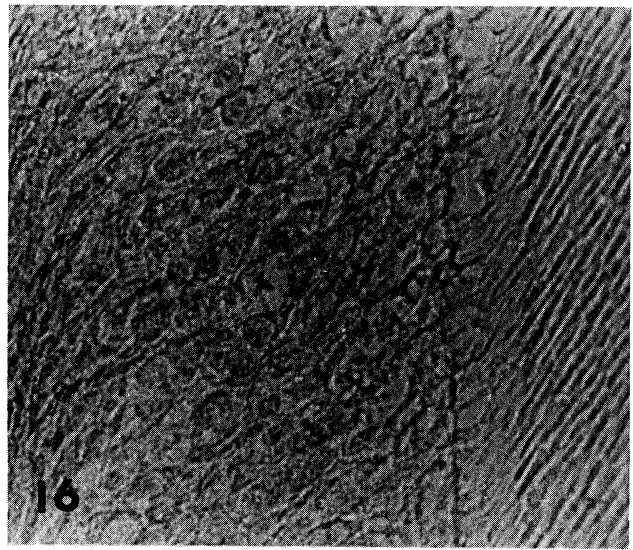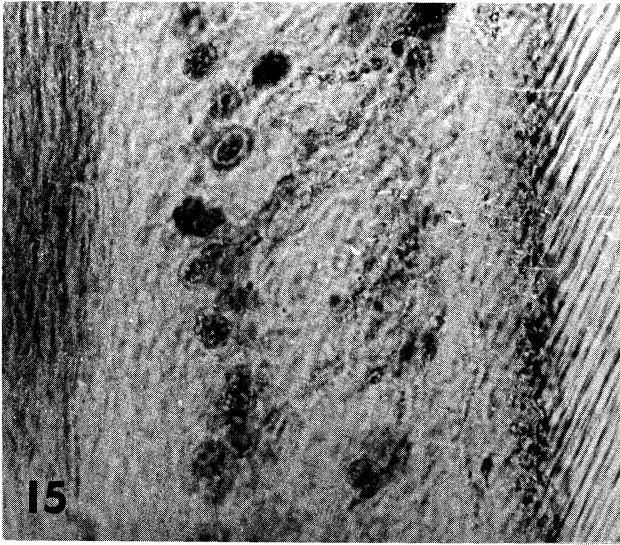
EXPLANATION OF FIGURES

Fig. 19   A section through the middle third of a control rabbit incisor.
           Succinic dehydrogenase activity is seen as a dark diaformazan de-
           posit along the odontoblasts. There is scattered activity through-
           out the pulp. Succinic dehydrogenase. 40 microns. X 140.

Fig. 20   A section through the middle third of a I.A.N. rabbit incisor (19
           days). There appears to be an increase in density along the
           odontoblast zone. Succinic dehydrogenase. 40 microns. X 160.

Fig. 21   A section through the middle third of an S.C.G. rabbit incisor with
           a relative intense reaction along the odontoblasts. Succinic de-
           hydrogenase. 40 microns. X 160.

Fig. 22   A section through the middle third of an I.A.N.-S.C.G. rabbit in-
           cisor. There is a dramatic increase in the diaformazan deposit on
           the odontoblast zone as well as in the pulp. Succinic dehydrogenase.
           40 microns. X 160

EXPLANATION OF FIGURES

Fig. 23   A longitudinal section towards the tip of a control rabbit incisor.
           There is an even layering of dentin with closure of the pulpal canal
           at the tip. Rowle's silver stain. X 20.

Fig. 24   A longitudinal section through the tip of a I.A.N. rabbit incisor
           (19 days). The pulpal canal has not completely closed. Some small
           vessels may be seen within as well as some distortion of the new
           dentin. Rowle's silver stain. X 20.

Fig. 25   A longitudinal section through the tip of a S.C.G. rabbit incisor
           (19 day). There is little change in the new dentin. Rowle's silver
           stain. X 20.

Fig. 26   A longitudinal section through a I.A.N.-S.C.G. rabbit incisor (19
           days). The new dentin is gnarled and distored with pockets of im-
           perfections. The incisal canal remains open with sloughed off pulp
           cells filling the canal at the tip. Rowle's silver stain. X 20.
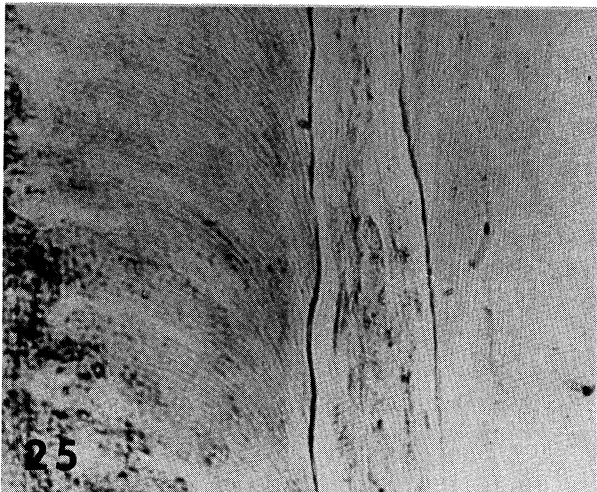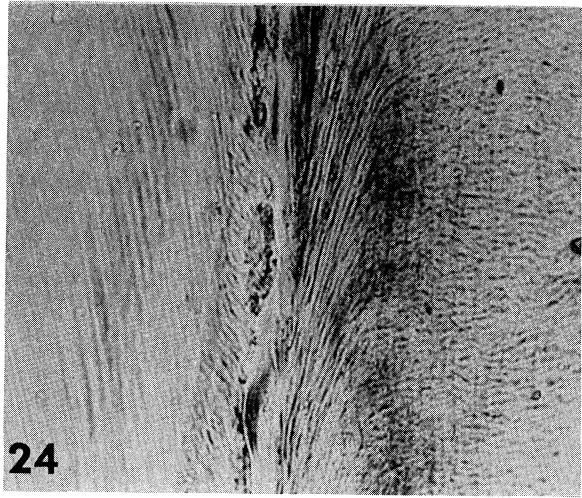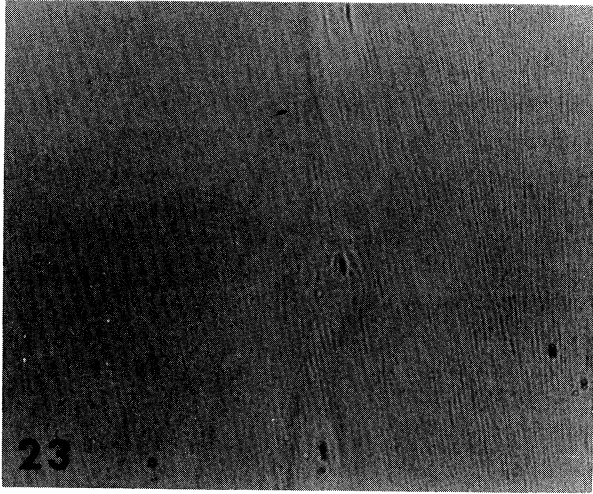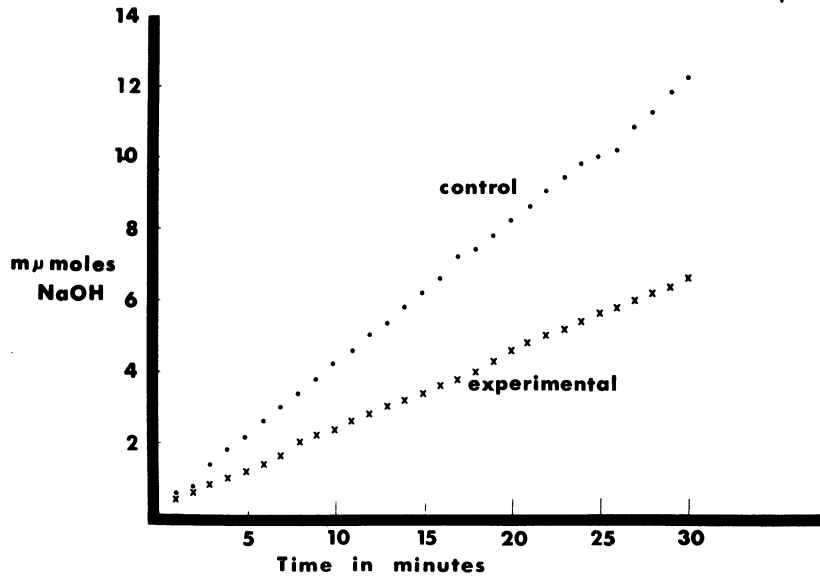
EXPLANATION OF FIGURES

Fig. 27   C-5 represents a random titrimetic run of the I.A.N. and a control.
           A total of 12.70 mμ moles was titrated for the control pulp homog-
           enate while 6.80 mμ moles of experimental pulp were titrated. This
           represents a significant reduction of acetylcholinesterase in the
           resected pulp homogenate which reacts with the acetylcholine sub-
           strate.

Fig. 28   Removal of the superior cervical ganglion has the least effect upon
           the concentration of cholinesterase in the computed significance
           reveals a P < .40 indicating a very slight, if any, reduction of
           total acetylcholinesterase activity.

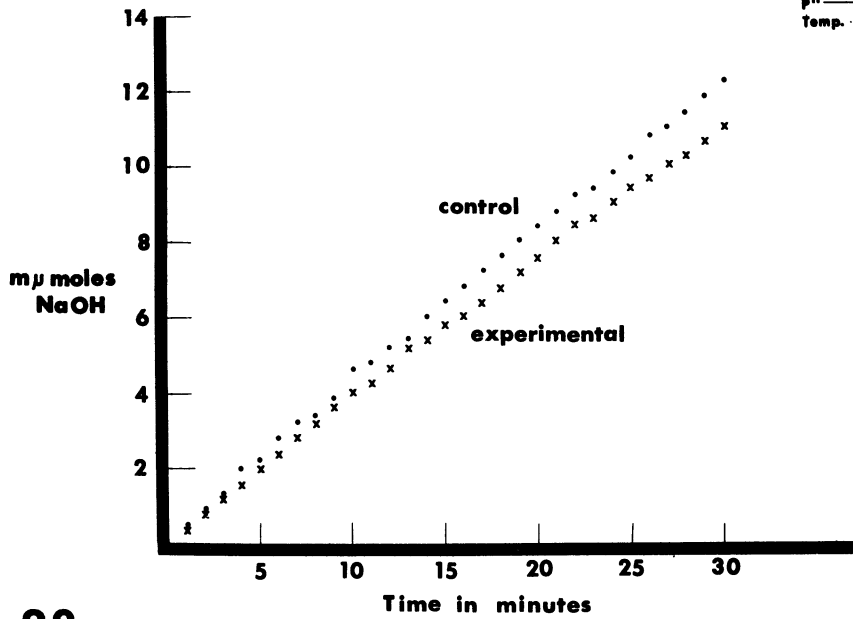## C-5 Inf. ALv. Nerve

pʰ   8.0
Temp. 25° C

mμ moles
NaOH

control

experimental

Time in minutes

27

C-2 Sup. Cerv. Ganglion

pʰ ———— 8.0
Temp. — 25°C

mμ moles
NaOH

control

experimental

Time in minutes

28

27

EXPLANATION OF FIGURES

Fig. 29   Removal of both the superior cervical ganglion and the inferior
           alveolar nerve results in a significant decrease in concentration
           cholinesterase in the incisor pulps.

Fig. 30   Acetylcholinesterase activity of individual runs of I.A.N., S.C.G.
           and a combination one compared to controls. The figure for each
           run represents the computed equivalent of mµ moles of NaOH titrated
           against the total volume of acetylcholine substance. The differ-
           ences between the paired control and experimental homogenates are
           noted with their total mean computed to demonstrate significance of
           the differences.

C-12, I.A. Nerve & S.C. Ganglion

p^h —— 8.0
Temp. — 25° C

control

experimental

mμ moles
NaOH

Time in minutes
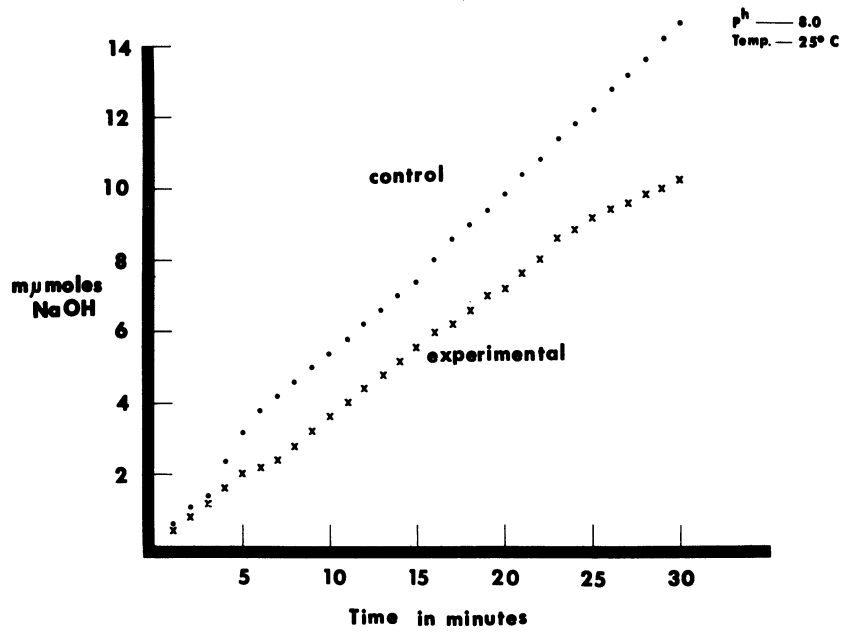
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ACETYLCHOLINESTERASE ACTIVITY
(mμmoles/min/mg tissue)

| Inferior Alveolar N.			Superior Cerv. Gang.			Inf.Alv.N.+ Sup.Cerv.G.		
Control	Experm.	Diff.	Control	Experm.	Diff.	Control	Experm.	Diff.
5.7	4.6	1.1	4.1	3.7	0.4	4.9	3.4	1.5
6.1	5.2	0.9	4.5	4.3	0.2	4.4	3.8	0.6
4.1	3.6	0.5	3.5	2.9	0.6	4.9	4.5	0.4
4.1	2.2	1.9	3.0	3.4	0.4	3.4	3.1	0.3
Mean		1.1			0.2			0.7
Significance		P <.03			P <.40			P <.25

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Maintenance of Viability of Odontoblasts in vitro

The purpose of this aspect of this study was to devise a technic of maintaining identifiable odontoblasts in vitro. Initial attempts utilized cell culture technics to obtain odontoblasts. The pulps were removed from recently extracted teeth leaving odontoblasts and a layer of pulpal tissue along the dentinal wall. Dentinal chips and fragments of crowns with adherent cells were placed in various types of media. Odontoblasts did not proliferate on coverslips in cell culture, and both odontoblasts and pulpal tissue underwent rapid degeneration and necrosis in tissue culture. Several explanations can be hypothesized for the failure of these technics. In cell culture the technic of obtaining dentinal chips necessitated injury to the odontoblastic processes, and odontoblasts, as post-mitotic cells, may have been incapable of the divisions required in cell culture. The majority of teeth used in this experiment were from older patients. Thus, the odontoblasts may have been in a quiescent state and rendered less capable of adapting to the conditions imposed by the tissue culture environment.

Somewhat better results were found when organ culture technics were used to maintain odontoblasts from the erupted molars of young mice. The teeth were extracted and a series were placed in three types medias for periods up to six days. The degenerative changes observed in odontoblasts and pulpal tissue became more extensive with time. Odontoblasts demonstrated a loss of cytoplasmic basophilia, then intercellular spacing. decrease in cellular height, cytoplasmic vacuolation, distal cell rounding, and finally detachment from the dentin, rounding the cellular death. Pulpal tissue demonstrated early vacuolation which may have given the appearance of a decrease in the number of cells and intercellular fibers. Degeneration and necrosis occurred first at the apex, then progressed into the crown. Necrosis was fairly complete by the fifth or sixth day of culture. Odontoblasts appeared to be more resistant than pulpal tissue to degeneration and necrosis.

Recent results are encouraging when younger animals are used. Unerupted molars of one and two day old mice maintained up to five days in vitro demonstrated early degenerative changes of the enamel organ, however, little change in the odontoblastic cell layer or pulpal tissue. Odontoblasts maintained cytoplasmic basophilia and intercellular relationships, however, they demonstrated a decrease in height. The pulpal tissue demonstrated little alteration in morphology. It was apparent that organic dentinal matrix was deposited in vitro. Also, the fairly smooth outline of the matrix became roughened with additional invaginations with longer periods of culture (note figure). Organ culture with the younger tooth buds maintained intercellular relationships, minimized cell injury, did not encourage cell proliferation, and allowed identification of odontoblasts by location and morphology.
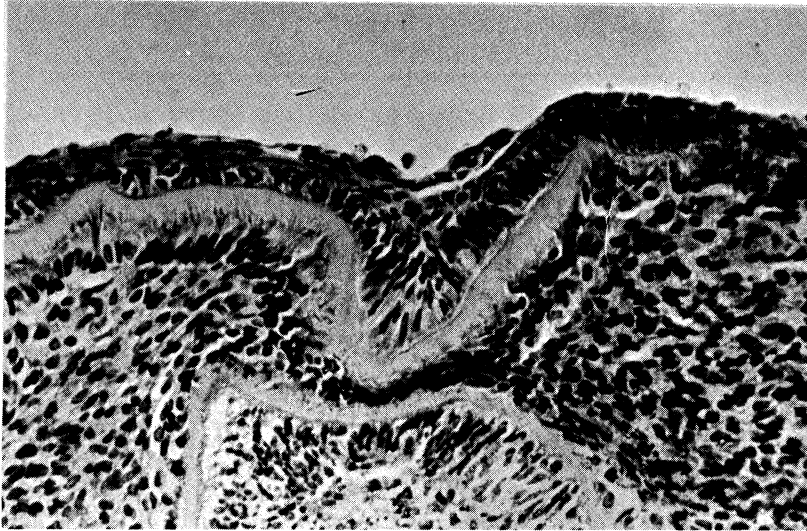
One purpose of developing the organ culture of young tooth buds is to maintain the viability of the odontoblasts for electrophysiological studies. Microelectrodes will be inserted in these odontoblasts to test for the presence of electronic junctions. Another reason for studying the odontoblasts in the tissue culture environment is to study the cell when released from possible control and regulation of the central nervous system.

Neural Elements in Developing Mouse Molars

Attempts to account for pain conduction in the dentin had led numerous investigators to pursue several theories of pain transmission in the tooth. The theory with the most support is based on direct innervation of the dentin by nerves which elicit painful responses when stimulated, but the extent and distribution of nerves in predentin and calcified dentin remains to be clarified. Arwill (1958) used Palmgren's silver staining method and Hansen's iron trioxyhaemateine and observed that no nerve fibers could be traced into developing dentin of teeth of 13-32 week old human fetuses nor into mineralized dentin of erupted human teeth. Nerve fibers could occasionally be observed in the matrix of predentin but did not accompany the odontoblastic processes into the tubules. Nerve fibers could nowhere be found in intimate contact with odontoblasts. Avery and Rapp (1959), Zerosi (1959), Kerebel (1964), and Bernick (1964) observed only incidental nerve fibers in predentin and none in calcified dentin, but recently several investigators such as Hattasy (1961), Fearnhead (1961), and Stella and Fuentes (1961) have claimed light microscopic evidence of nerve fibers in the dentin.

Ultrastructural investigations of nerves in dentin have not clarified the controversy because Arwill (1958) denied the presence of nerves in dentin after an extensive survey of many sections, whereas Frank (1966) reported what he believes to be unmyelinated nerve fibers to be intratubular in location. Frank characterized the fibers to be 0.5 to 0.7 μ in diameter and lying adjacent to the odontoblastic processes within the dentinal tubules. These structures appeared to contain mitochondria and several small vesicles. Minute neurofibrils were not observed in the nerve fiber and it appeared free of the Schwann cell covering. Since Frank revealed only one photograph of a possible nerve fiber, doubt still remains regarding the true nature of this structure as a nerve fiber, its frequency, distribution and how for it extended into the dentin.

Arwill (1967) reported an investigation of the pulpo-predentin region of human premolars (12-13 years) in which he split the teeth and removed the pulp and predentin with a sharp spoon excavator. The specimens were surveyed with the electron microscope, and Arwill reported structures which he labelled "associated cells" (AC) in close apposition to 42.5% of the odontoblastic processes in the predentin. The associated cells which contained heavily stained cellular components were separated from the odontoblastic processes by a triple-layered membrane and a gap. The AC were filled with round or oblong vesicles (250-700 Å) containing a granulated, dense substance. In the cytoplasm of the AC there were dark, opaque bodies 500-1500 Å in diameter. The sizes of the AC varied from 0.5 μ to 1.6 μ in diameter and occasionally several AC accompanied one odontoblast process. Arwill (1967) hesitated to call the associated cells

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nerve fibrils, but he equated the size of the vesicles present in the AC to synaptic vesicles and the gap between the AC and the odontoblastic processes as similar to the "synaptic cleft" as described by DeRobertis in (1964).

Since nerves do not appear to exist in young dentin of newly erupted teeth, and nerves have never been demonstrated to approximate the dentino-enamel junction, alternate conductive pathways in the dentin not based on the presence of nerves have been considered. Brannstrom (1962) proposed a hydro-dynamic mechanism in the transmission of pain-producing stimuli through the dentin. The theory attempts to account for the sensitivity of exposed dentin to varied stimuli such as heat, drying by air, negative pressure (Brannstrom, 1962b, 1960a, 1960b, 1962c). Such stimuli were considered to cause the displacement of the odontoblastic tubular contents and aspiration of the odontoblastic nuclei into the tubules. Brannstrom (1962c) postulated that if the displacement of the tubular contents occurred rapidly enough, deformation of the nerves may occur in the pulp or predentin and damage to the odontoblasts may result in a sensation of pain. When aspiration of odontoblasts occurred in teeth in which no pain was felt, Brannstrom speculated that the rate of movement may have been too slow to act as a stimulus. Kramer (1955) found that although odontoblastic aspiration occurred after certain operative procedures, no correlation was observed between those teeth in which pain was felt during the operation and those exhibiting aspiration.

The odontoblastic process has been postulated to be capable of conducting an impulse from the dentino-enamel junction of the cellular body of the odontoblast where synaptic-like junctions occur with nerve endings in the pulp (Avery and Rapp, 1959, and Rap, Avery, and Strachan, 1967). This theory is based on the localization of the enzyme acetycholinesterase in the odontoblastic process and in the area of the odontoblast-pulpal nerve ending. The stimulation of a single odontoblastic process could cause excitation of many neighboring processes since they are connected by an intricate network of minute lateral branching processes (Avery and Rapp, 1959). Kroeger (1963) utilized this technique and observed a localization of cholinesterase in the rat incisor odontoblasts and odontoblastic processes. Ten Cate and Shelton (1966) using the direct coloring method of Karnovsky and Roots (1964) for localization of cholinesterase disputed the localization of the enzyme in the odontoblast and in the odontoblastic process. Rapp, Avery, and Strachan (1967) noting cholinesterase in the odontoblast with this technique reasoned Ten Cate and Shelton may have utilized sections which were too thin or teeth possibly too young to exhibit the localization of cholinesterase.

A review of the several theories of pain transmission in teeth included above has revealed a need to resolve the role of neural elements, the odontoblast and the odontoblast processes in pain transmission in the tooth.

The specific aims of the histological and ultrastructural portions of this investigations are:

1. To establish if nerve fibers develop in the dentin of developing teeth and at what ages such nerve fibers first appear. The frequency of distribution and the distance that nerve fibers extend toward the dentino-enamel junction would establish the extent of the innervation of the dentin and the predentin.

2. To describe the fine structure of developing odontoblasts and their processes and to observe their structure for possible sensory receptors which serve as intermediate conductive pathways between the dentino-enamel junction and the pulpal nerves.

3. To observe the relation of the free nerve endings of the pulpal nerves to the odontoblastic layer.

4. To study the changes in the morphology of the odontoblastic process as it extends from the apex of the odontoblast to the dentino-enamel junction.

METHOD AND MATERIALS

In order to facilitate the search for nerves in the predentin and dentin by ultrastructural methods, it was first necessary to observe the sequential development of nerves utilizing the developing mouse molars. A light micro-scopic study was undertaken of the developing molars of mice. Ninety-four Swiss-Webster white mice were sacrificed by ether inhalation and decapitation at 3, 5, 9, 15, and 25 days of age. Each age group contained from 16 to 28 an-imals. The right halves of the mandibles were removed, and portions of the mandible mesial to the first molar and distal to the third molar were excised to reduce the size of the specimen. The tissues were fixed at 4°C from 12-36 hours in 10% formalin in a 0.2M phosphate buffer at pH 7.4, in Carnoy's acid alcohol, and in Hofker's acid alcohol. Following fixation the specimens were washed and then decalcified in 4 N formic acid from 24-120 hours at room tem-perature. The specimens were then washed and dehydrated by passage through ascending grades of ethanol and double embedded in parlodion. After infiltra-tion with parlodion, the specimens were cleared in benzene and embedded in paraplast. The tissue specimens were oriented for longitudinal, mesio-distal sections of the mandibular right first molar. Serial sections were mounted from a gelatinized water bath and allowed to stand overnight at 37°C.

Acid alcohol fixed specimens of each age group were treated with col-lagenase and then silver stained. One half of the formalin fixed specimens in each age group were treated with collagenase and silver stained, while the other half received only the silver stain. Prior to collagenase digestion, all slides received a 0.5% celloidin coating to stabilize and affix the tissue sections to the slide. After receiving the celloidin coating, the sections were hydrated and thoroughly washed in distilled water and then placed in a horizontal Plexiglas slide carrier for incubation with collagenase. Approxi-mately 0.2 ml of 1% collagenase solution containing 0.067 M phosphate buffer

at pH 7.4, 0.01% CaCl$_2$, and 0.45% NaCl was placed on each slide with a pipette. Careful drying of the periphery of the slide with a tissue was sufficient to maintain the enzyme over the tissue sections. The slide carrier was then placed in a moist, covered staining dish for incubation at 37°C for 12-24 hours. With each slide carrier incubated with collagenase, one slide with enzyme and one slide with buffer solution were stained with Masson trichrome or Van Gieson stain to judge the degree of enzymatic digestion. Following incubation, the slides were washed in distilled water and then post-fixed in 10% buffered formalin for 12-18 hours at 4°C prior to silver staining. The Ungewitter silver stain as modified by Rowles and Brain (1960) a 2-hour protargol silver stain, and a silver cyanate stain were used in this project.

The photomicrographs were taken on a Zeiss photomicroscope with an auto-matic exposure setting device. Panotomic-X 35 mm film was used with magnifica-tions of 113, 288, and 720 times before enlarging to four- by five-inch prints. Enlargement and printing of the negatives were done on Kodak F-2 single weight medalist paper.

In the ultrastructural investigation of developing mouse molars, initial fixation with 2% glutaraldehyde in a phosphate buffer and postfixed in 1% osmic acid produced serious artifacts as indicated by marked intercellular spacing of the odontoblasts, excessive swelling of cisternae of the endoplasmic retic-ulum, and disruption of cellular and mitochondrial membranes. In subsequent periods of fixation, 5-10 mandibular first-molars from 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 18-day old Swiss-Webster mice were fixed in 1.33% osmic acid buffered with s-collidine at pH of 7.4 with 6% sucrose added to obtain an iso-tonic solution. The teeth were fixed for two hours at 4°C, washed in distilled water, dehydrated in a series of graded ethyl alcohols, held in two separate changes of propylene oxide, transferred to 50% propylene oxide and 50% Epon 812, and finally embedded one hour later in Epon 812 in Beam Capsules. The blocks were placed in an oven at 37°C overnight, 45°C for the following day, and in a 60°C oven for the next night. The blocks were trimmed and sectioned on an LKB ultramicrotome equipped with a DuPont diamond knife. The sections were stained with a 2% aqueous solution of uranyl acetate for 20-30 minutes, and some sections were stained with 2% potassium permanganate for 20 seconds, washed thoroughly and dried. Over 1500 photographs were taken of the sections with an RCA-EMU 3D electron microscope and printed on Kodak photographic paper.

RESULTS

In the histological portion of this investigation, the silver staining was combined with collagenase digestion to establish the development of nerves at various ages in the developing mouse molars. Several distinct patterns of development were observed chronologically (Fig. 8). The first important ob-servation of neural development appeared at three days when isolated nerve fibers were present in the basal portion of the pulp (Fig. 1) and appeared to

be associated with blood vessels. At five days the nerves were developing rapidly and approaching the odontoblastic layer (Fig. 2). By nine days, nerve fibers were evident in the odontoblastic layer. By this time enamel formation was completed and root formation was well established (Fig. 3). At nine days the rate of deposition of dentin appeared significantly decreased. By 15 days, a subodontoblastic nerve plexus was present (Figs. 4 and 5) and eruption of the tooth was imminent. From 15-25 days, the nerves increased in density but no connections between nerve fibers and odontoblasts were evident. Nerve fibers were not observed in dentinal tubules or calcified dentin up to 25 days (Fig. 6), although an occasional loop of nerve tissue may have become entrapped in the predentin by 25 days (Fig. 7).

With the histological information provided by the light microscopic study of the development of nerves in the pulp of mouse molars, it was possible to begin a search for nerve fibers in the dentinal tubules and surrounding the odontoblastic layer with the electron microscope. In a survey of dentin of mouse molars of 1-18 days, no identifiable nerve fibers were observed in the dentinal tubules or predentin (Figs. 9 and 10). From 11-18 days, more observations must be recorded, especially at higher magnifications, to verify the presence of nerve fibers but the present progress has not revealed any nerve fibers in the dentinal tubules. Progress in sectioning calcified dentin was difficult because of the lack of sufficient diamond knives, but presently an adequate supply has speeded progress of sectioning the more mature teeth.

In the pulpal tissue of nine day molars, large nonmyelinated nerve fibers were easily identified in the subodontoblastic layer (Fig. 11). These nerves were composed of several axons surrounded by Schwann cell bodies, and several smaller nerve fibers appeared to be free of the Schwann cell covering. The axons contained minute neurofibrils, small mitochondrial-like organelles, and occasional small synaptic-like vesicles. Interspersed among the cellular processes of the subodontoblastic layer were observed small structures which resembled small nerve fibers with a central axon and a Schwann cell covering (Fig. 12). No free nerve endings have been observed to end directly upon the odontoblasts as observed in the acinar cells of salivary glands (Han, 1968).

In developing mouse molars significant changes occur in the odontoblastic processes as they extend out from the apex of the odontoblasts toward the dentino-enamel junction. In the predentin, the processes contain numerous granules which exhibit a variety of form and consistency (Figs. 13-15). Small vesicles are visible in the odontoblastic processes in the predentin (Fig. 10). Small isolated vesicles or vacuoles are observed occasionally in the processes which extend into the calcified dentin, but the granules disappear once the odontoblastic processes are observed in the dentin. Small branches are observed extending from the odontoblastic processes into the predentin (Figs. 13 and 15), but many of the smaller lateral branches appear to be obscured in the calcified dentin. During the initial stages of dentinogenesis, the distal tips of the odontoblastic processes appear almost in contact with the smooth membrane which delineates the future dentino-enamel junction.

The apex of the odontoblast exhibits a zone which contains granules and vesicles but relatively free of mitochondria and endoplasmic reticulum. Basal to this zone numerous mitochondria appear interspersed among narrow profiles of rough-surfaced endoplasmic reticulum (Fig. 16) which is also observed densely in the perinuclear and in the basal portion of the odontoblast (Fig. 17). An extensive Golgi apparatus is located in a supranuclear position and is composed of numerous small vesicles, elongated flattened profiles, and several dilated vacuoles (Fig. 18). The structure of the cellular body of the odontoblast does not appear to change from the first day through the ninth day. Insufficient sampling of older teeth limits further description of the odontoblastic changes after nine days.

Tight junctions were observed frequently between the odontoblasts and usually located in the distal one-quarter of the lateral cell membrane. Tight junctions appeared at one day to be both in the formative stage and already mature (Fig. 20). They were observed consistently between odontoblasts in all the teeth examined (Fig. 21). Tight junctions appear in approximately the same position between all of the odontoblasts regardless of the age of the tooth (Fig. 19). Any differences in length of the tight junctions in the various age groups are yet to be measured.

DISCUSSION

The transmission of painful stimuli in the dentin by nerve fibers in the dentinal tubules remains an important theory of pain conduction in teeth. Histologists differ on the presence of nerve fibers in the dentin, and ultrastructural investigations have left unanswered the presence of nerve fibers in the calcified dentin. Arwill (1958) denied the presence of nerves in the predentin or dentin, but Frank (1966) reported what may have been a nerve fiber in the dentin of a human tooth, but he included no discussion of the extent or distribution of nerves in the dentin. Arwill (1967) utilized improved methods of sectioning and reported numerous "associated cells" accompanying 42.5% of the odontoblastic process in human predentin and compared the morphology of these structures to the nerve fibers. Several important findings of our investigation are pertinent in interpreting Arwill's findings. The nerves of developing mouse molars (1-25 days) were observed at various stages of development of the molars and it was apparent that prior to nine days, no nerves had penetrated the odontoblastic layer. An extensive survey of the same ages (1-9 days) of developing molars was conducted with the electron microscope and no nerve fibers were observed in the predentin or in the calcified dentin. Such evidence indicates that nerve fibers will not reach the dentino-enamel junction. An investigation of dentinogenesis in rats (Reith 1968), 7-10 days did not reveal nerves in the predentin. Continued investigation of older mouse molars is necessary however, to establish if nerves are present in the calcified dentin. It will be of interest to determine if the structures in the predentin reported by Arwill (1967) as "associated cells" are present in the

mouse and if nerves as reported by Frank (1966) are in the dentin. With the improved methods of sectioning of calcified tooth tissues, the presence and distribution of nerve endings in dentin will be an important part of our investigation.

A second important finding of this investigation was the uniform presence of tight junctions between odontoblasts of developing mouse molars. These intercellular attachments have been observed in many types of tissues such as epithelium (Farquhar and Palade, 1963), smooth and cardiac muscle (Dewey and Barr, 1964), and neural tissue (Robertson, 1961). Both Arwill (1967) and Reith (1968) reported intercellular junctions in the odontoblastic layer, but Reith did not consider the tight junctions as a conspicuous feature of the junctional complex. The significance of the tight junction in the odontoblast is yet to be established, but it has been demonstrated physiologically in other types of tissues that the tight junctions constitute regions of reduced electrical resistivity than its neighboring cell membrane (Pappas and Bennett 1966). An additional functional aspect of the tight junction is its increased permeability which allows an intercommunicating system between neighboring cells in which ions and small molecules may move freely across from one cell to another with no appreciable leakage to the exterior of the cells (Loewenstein, 1966). If such functions operate for the odontoblastic tight junctions, these intercellular connections may serve as pathways of impulse transmission between odontoblasts and also may function in the regulation of the synchronous action of odontoblasts in the process of dentinal formation, especially when early dentinogenesis appears independent of direct innervation.

A final significant observation of the investigation is the changing morphology of the odontoblastic process from the apex of the odontoblast to the dentino-enamel junction. The granules and vesicles observed in the odontoblastic process of the predentin are absent in the odontoblastic process as it becomes incorporated in the calcified dentin. The differences in morphology of the odontoblastic process in the predentin and in the dentin may signify a difference in function of these two regions.

FUTURE INVESTIGATION

Continued investigation of the ultrastructure of the developing mouse molars must include additional observations of the dentin of older erupted teeth for nerve fibers. Time has not been sufficient to completely evaluate older teeth for the presence of nerves in the dentinal tubules. Histochemical localizations of acetylcholinesterase will be attempted in the odontoblastic process of the mouse. Concurrent with the search for nerves in the dentin of older teeth will be the observations of tight junctions between odontoblasts of the teeth of the older age groups following eruption. Finally, ultrastructural studies of human teeth of a variety of ages must be made for nerves in the dentin and the presence of intercellular connections (tight junctions) between odontoblasts. Of particular interest are human teeth which have been

erupted and in function after the completion of the root, since it has been postulated by Fearnhead (1961) that nerves are not observed in the dentin until the tooth has been in function for a period of several years after the root formation is completed.

The search for tight junctions between the odontoblasts of developing mouse molars and human teeth will continue since these intercellular connections may serve as pathways of impulse transmission and also may be important in the regulation of the synchronous action of odontoblasts in the process of dentin formation especially when early dentinogenesis appears independent of nerves.

Further investigations will also concern the effect of resection of either the inferior alveolar, cervical sympathetic or both nerve supplies in the New Zealand rabbit. Light microscope studies which are currently in progress have noted the changes in acetycholinesterase in the odontoblast after resection. Histological and histochemical changes after resection were also described. It is of interest to study ultrastructurally the entire odontogonic zone as well as the process of the odontoblast in the resected teeth. Again the effect of neural resection on dentin formation is of interest. Altered dentin formation after cervical sympathetic nerve resection was described as viewed by the light microscope. Further information concerning the ultrastructural aspects of this process will be studied. Also studies of the effect of preparation of cavities on normal teeth or those that have had their nerve supply resected are being carried out. Both the light and electron-microscope will be used to investigate any changes. It is not known what effect resection has on this defense mechanism.

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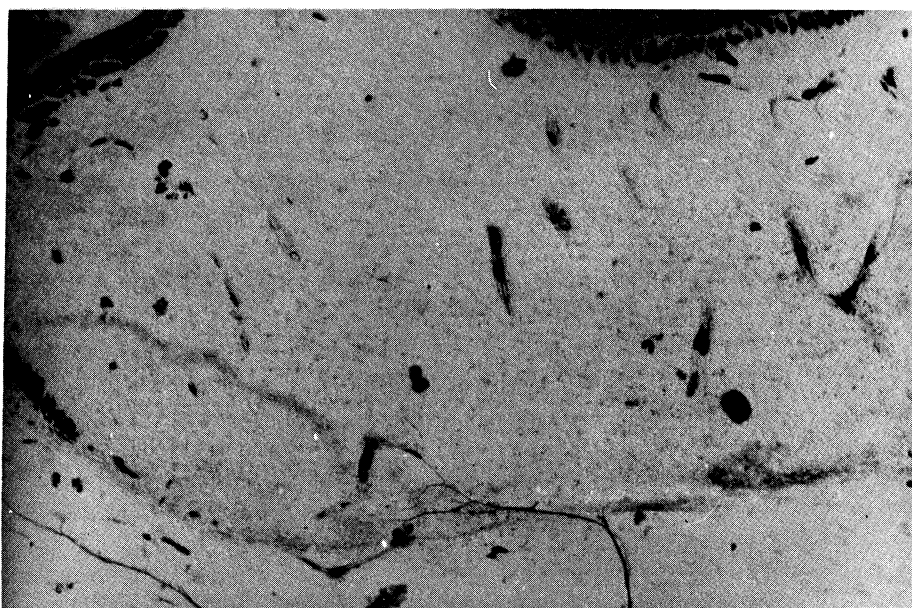
Fig. 1, 3rd day. Isolated nerve fibers in the
          basal portion of the dental pulp. Stained
          with silver cyanate. 288x.
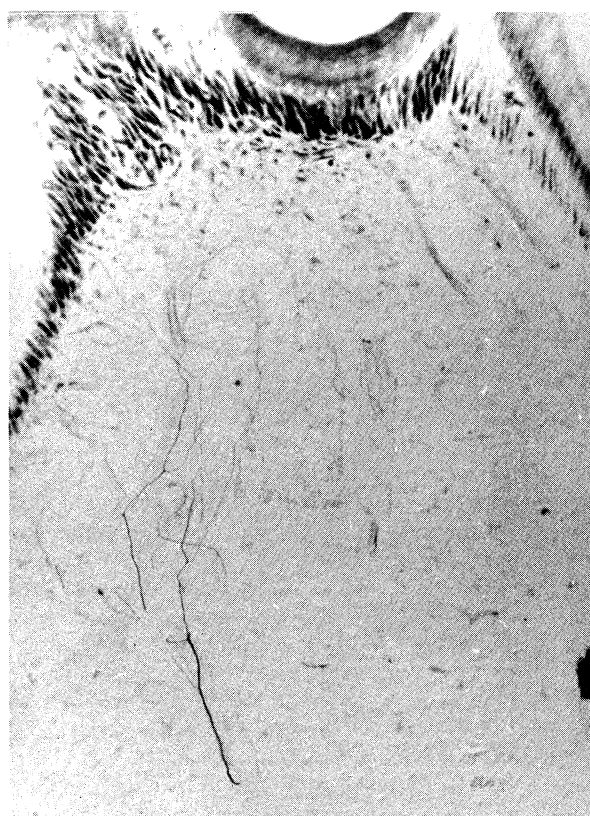
Fig. 2 Nerve fibers and blood vessel extending toward
        the odontoblastic layer. Stained with silver cyanate. 288x.
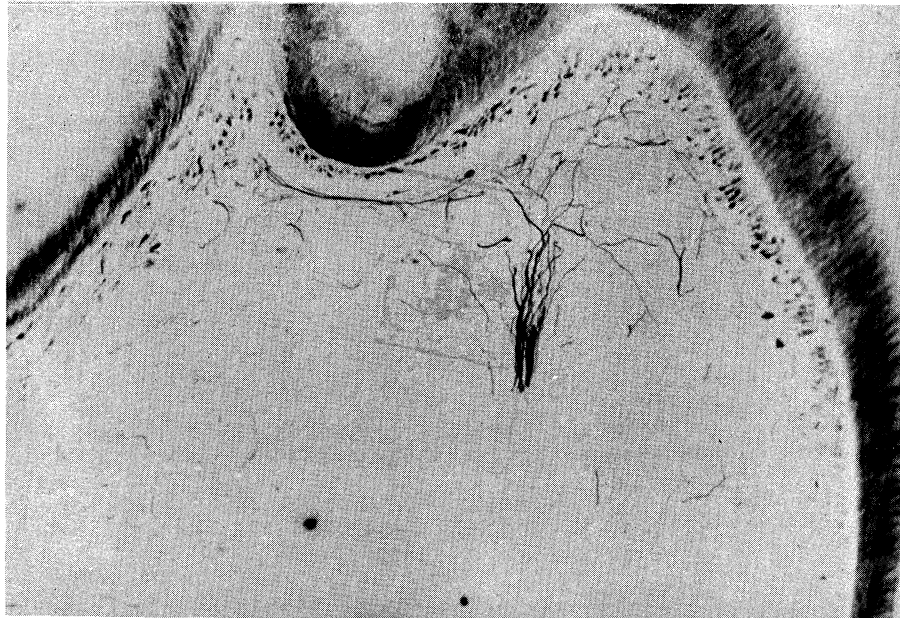
Fig. 3   9th day.   A main nerve trunk in the pulp horn and
         its branches reaching the odontoblastic layer.
         Stained with silver cyanate.   288x.
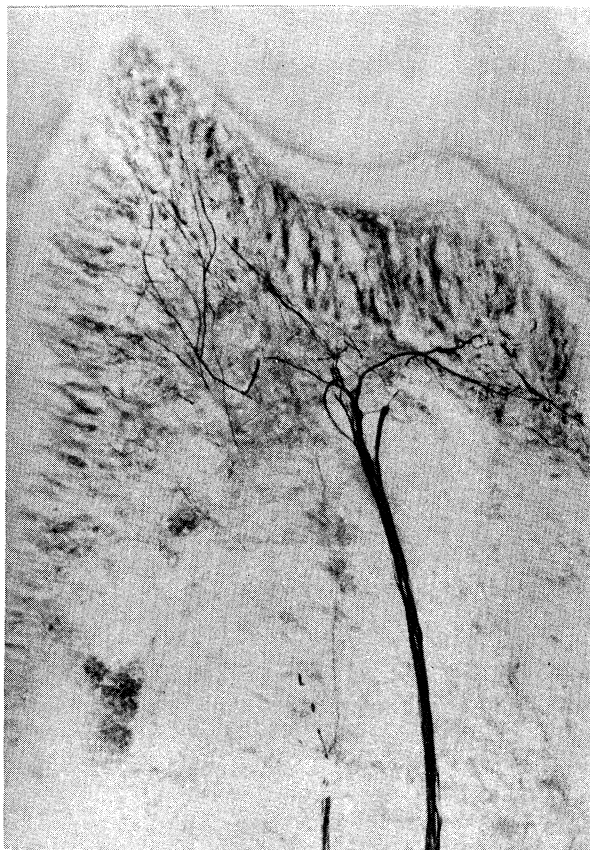
Fig. 4   15th day.   Nerve trunk in association with a blood
         vessel.   Partial collagenase digestion of the pulp
         has destroyed some pulpal cells and odontoblasts.
         Stained with silver cyanate.   288x.
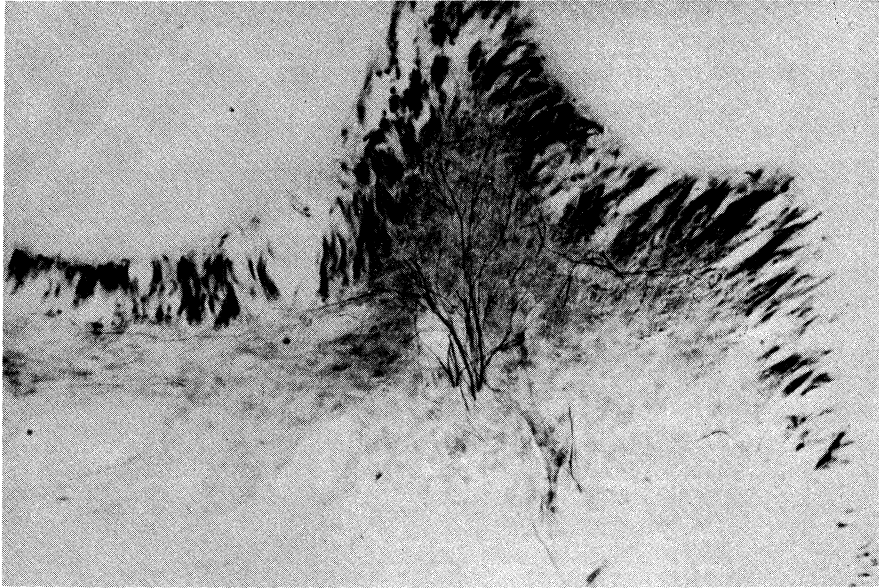
Fig. 5   15th day.   Collagenase digestion.   Early development of
a subodontoblastic nerve plexus.   Stained with the two
hour protargol silver stain.   288x.
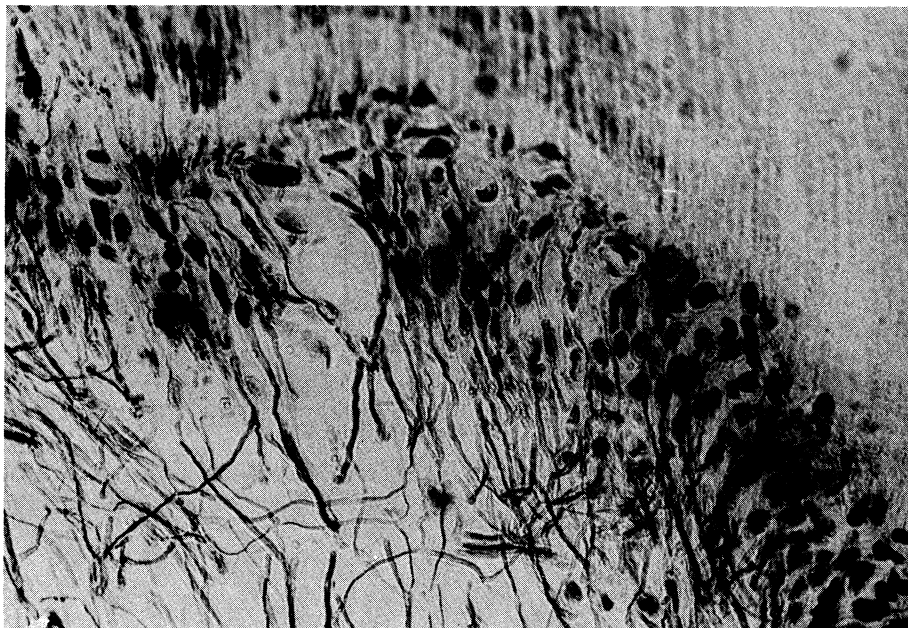
Fig. 6. 25th day.   Extensive branching of the nerve trunk can
be seen in the pulp horn.   An increased number of nerve
fibers are seen in the subodontoblastic nerve plexus.
Stained with silver cyanate.   288x.
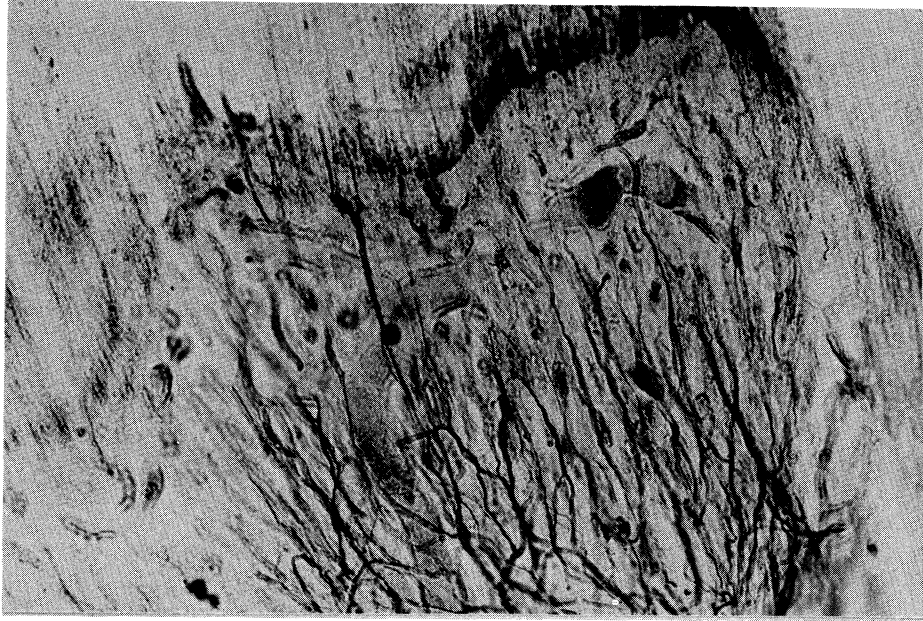
Fig. 7   25th day.   A single nerve fiber can be traced from
         the pulp into the predentin.   Stained with silver
         cyanate.   288x.
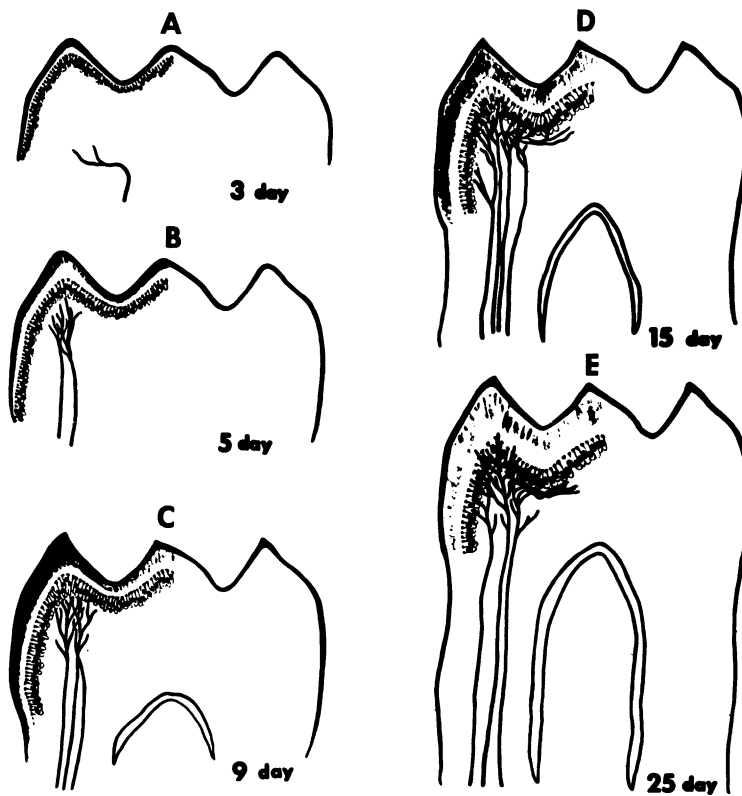
Fig. 8. A diagrammatic illustration of the development of nerves into the mandibular first molar of a mouse.

    A.   A three day molar in which the nerves are entering the apical pulpal tissue.

    B.   At five days the pulpal nerves have developed close to the odontoblastic layer.

    C.   By nine days smaller nerves have branched from the larger nerves and lie in the subodontoblastic layer.

    D.   At 15 days a subodontoblastic nerve plexus is evident.

    E.   By 25 days there was a noticeable increase in nerve fibers around the odontoblasts and several loops of nerve fibers were observed in the predentin.
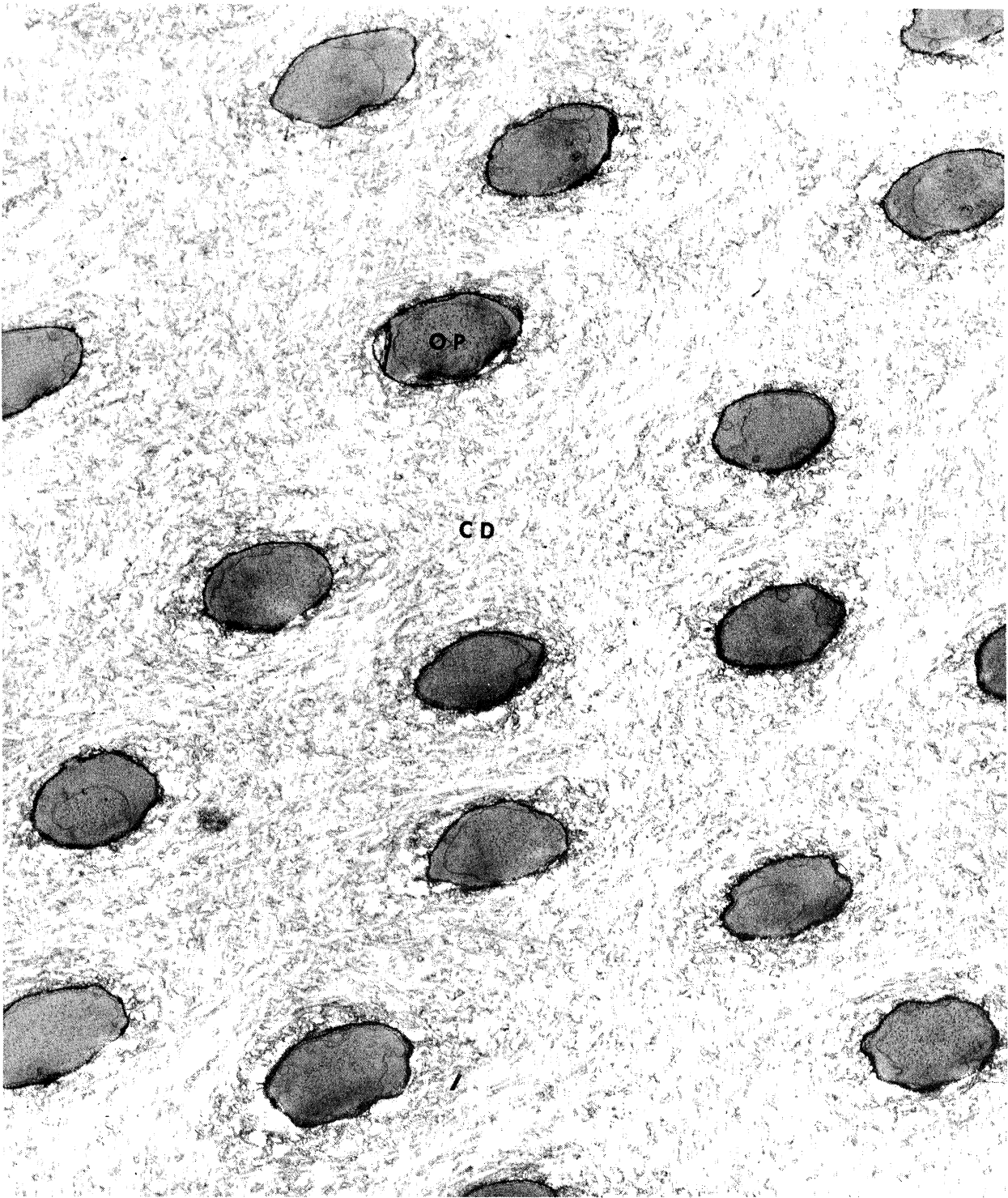
Fig. 9 A low power magnification of an area of calcified dentin
(CD) near the dentino-enamel junction of an 18 day old
mouse molar. The dentinal tubules contains single
odontoblastic processes (OP) which are almost devoid of
organelles. 13,500x.
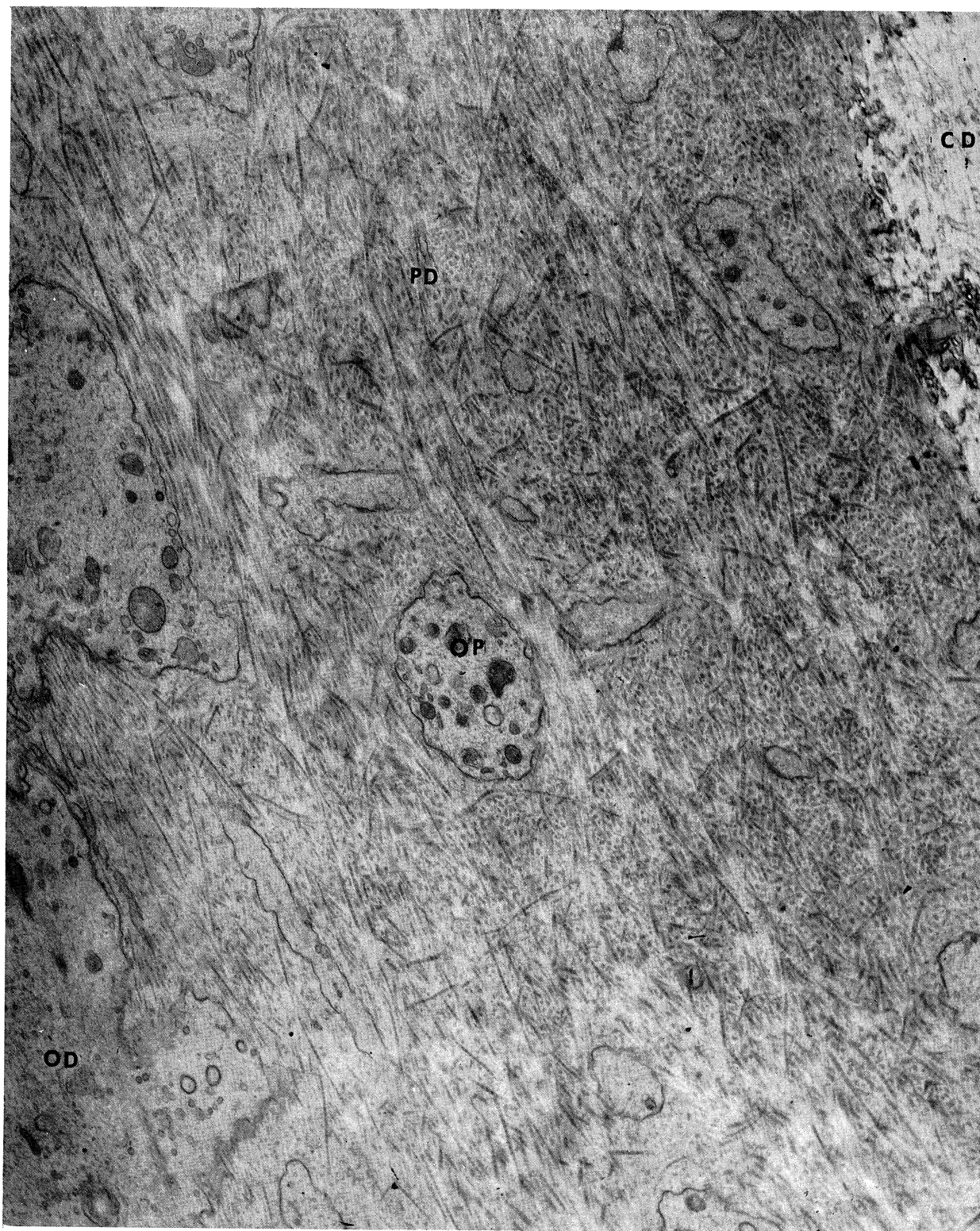
Fig. 10 A panoramic view of the apex of odontoblasts (OD),
predentin (PD), and calcified dentin (CD) of a nine
day old tooth. The odontoblastic processes contain
numerous granules and vesicles at the apex of the
odontoblasts and appear free of organelles as the
processes approach and enter the calcified dentin.
20,000x.
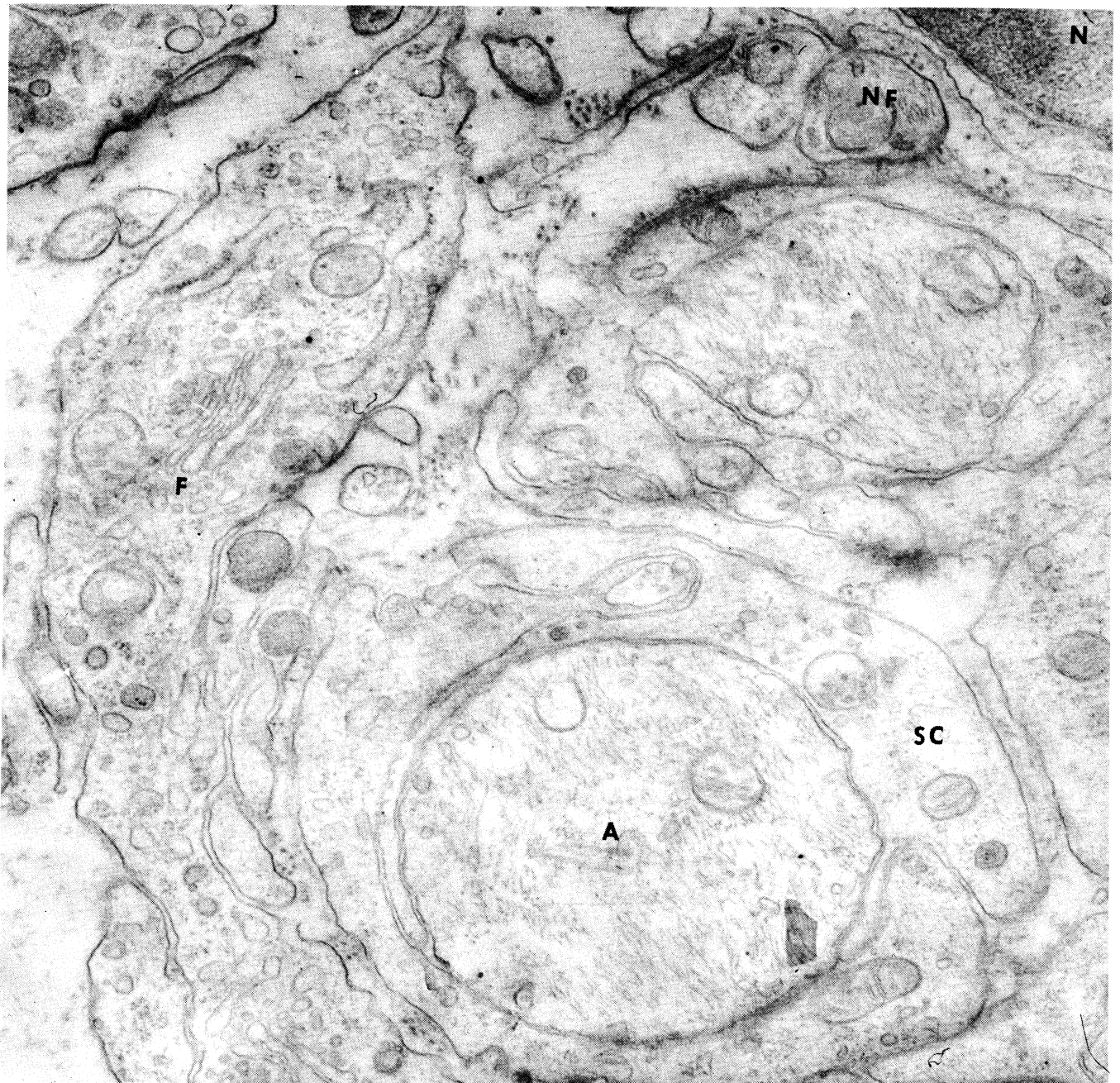
Fig. 11 A view of a small unmyelinated nerve of a nine day old
molar, in which the nerve is located in the subodonto-
blastic layer near the nucleus (N) of a endothelial cell.
The nerve consists of several axons (A) which contain
several mitochondria, numerous neurofibrils, and synaptic-
like vesicles. The axons are surrounded by a Schwann
cell covering (SC), and a small nerve fiber (NF) free of a
Schwann cell covering is located near the nucleus of the
endothelial cell nucleus (N). A process of a neighboring
fibroblast (F) is located to the left of the photograph.
41,500x.

Fig. 12 A small structure resembling a nerve fiber is located among cellular processes of the subodontoblastic layer and just basal to several odontoblasts (OD) in a seven day old tooth. The nerve contains a central axon (A) with one mitochondria, several vesicles, and neurofibrils, and is covered by a Schwann cell covering (SC). 42,500x.
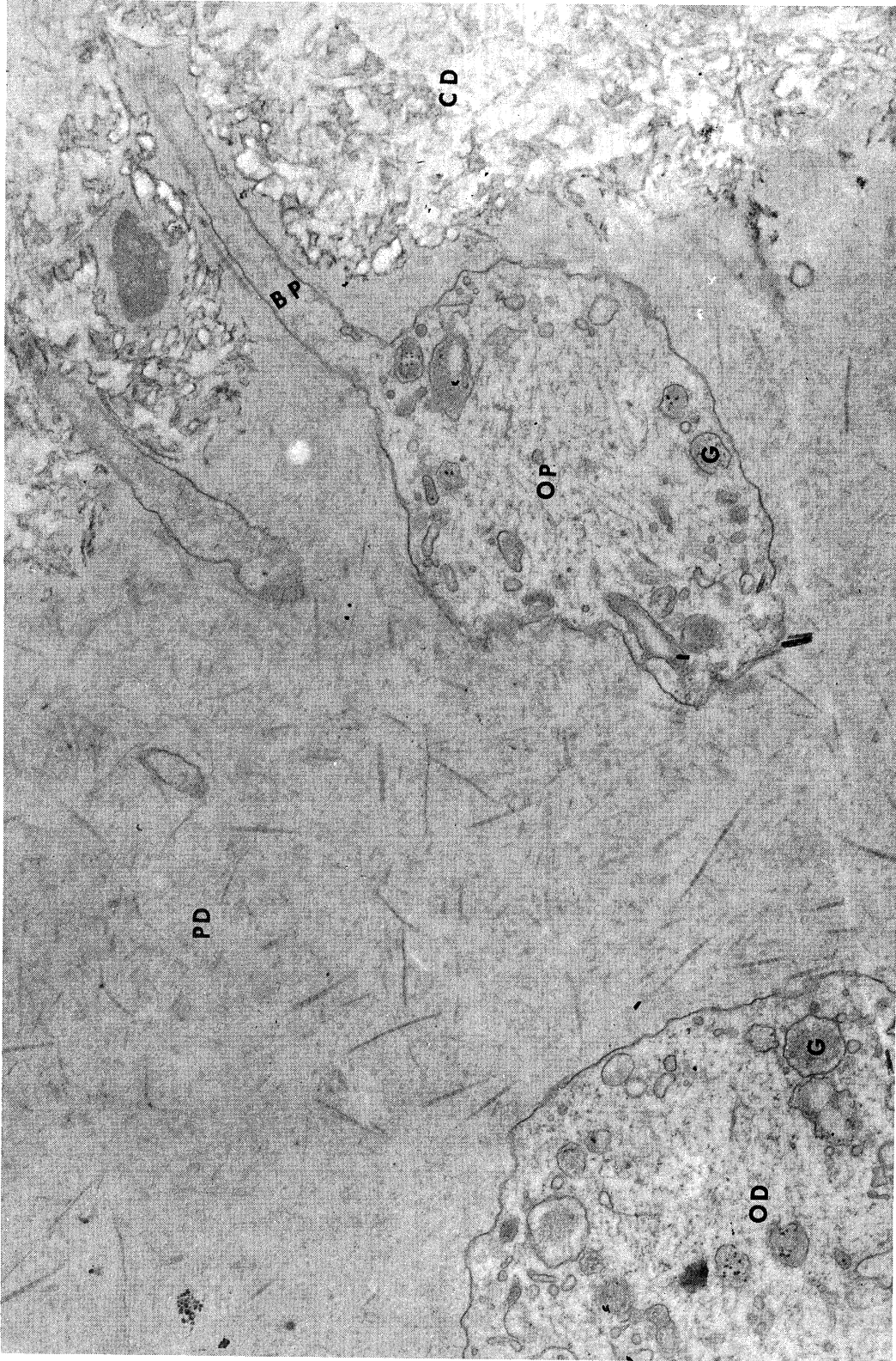
Fig. 13 A section through the predentin (PD) of a two day old mouse molar in which the apex of an odontoblast (OD) contains numerous granules and vesicles. A prominent odontoblastic process (OP) still containing granules and vesicles exhibits a branching process (BP) which is seen entering the calcified dentin (CD). 21,600x.
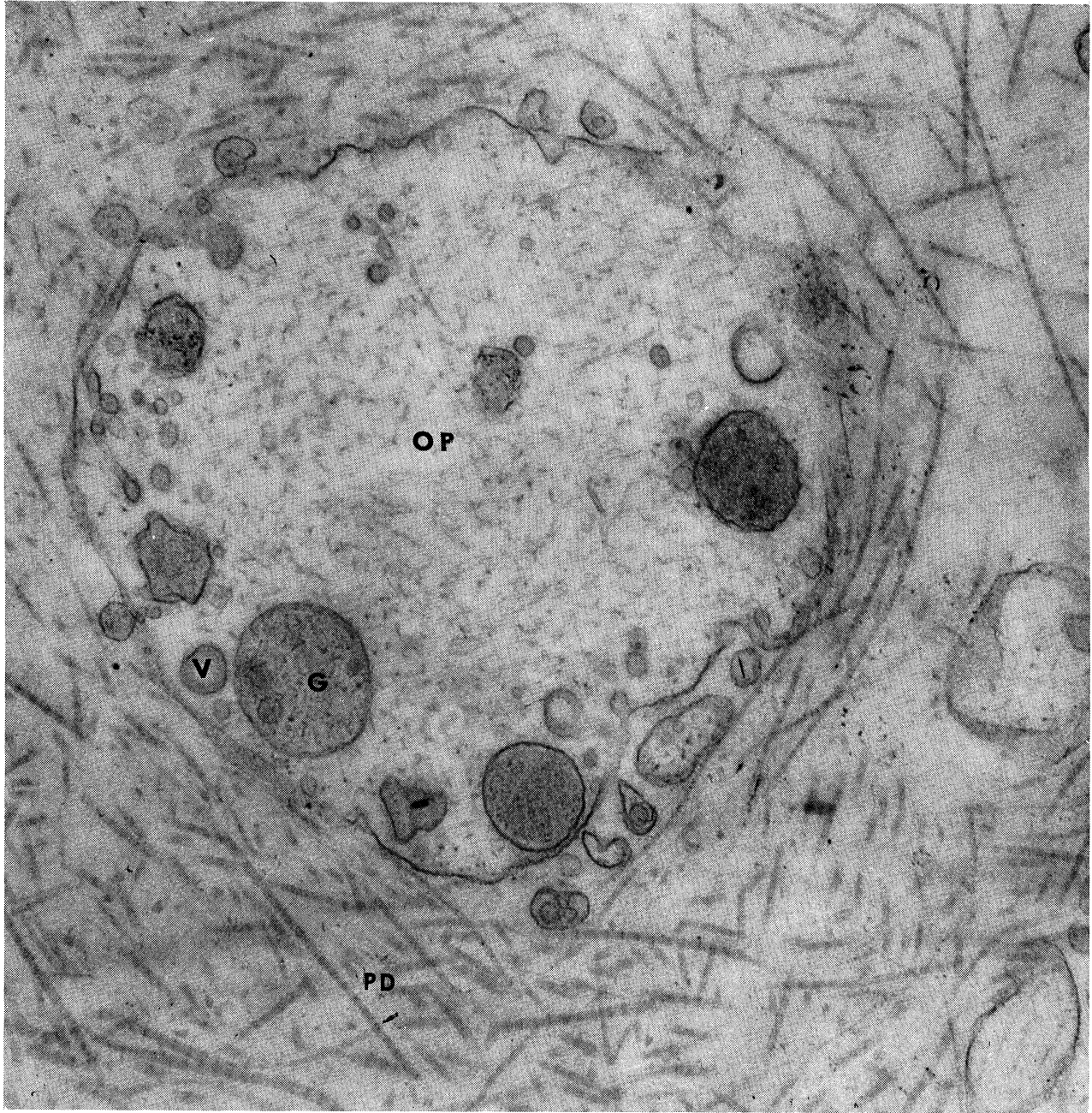
Fig. 14, A high power magnification of an odontoblastic process (OP) located
in the predentin (PD) close to the apex of the odontoblasts. The
process still retains granules (G) and small vesicles (V) in its
cytoplasm. 46,000x.
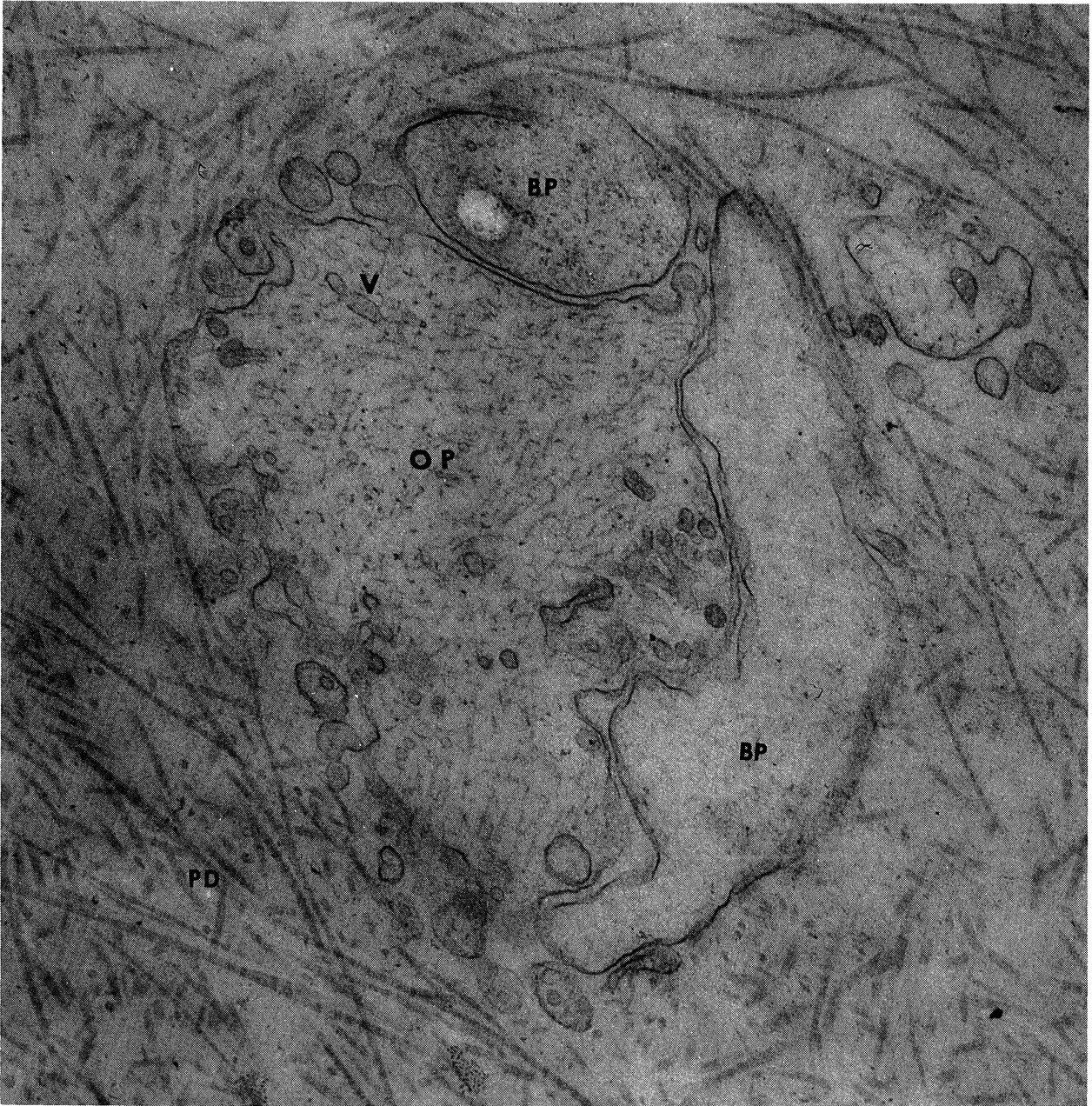
Fig. 15, A cross section of an odontoblastic process (OP) located deep in the predentin (PD). In addition to the main process (OP), several branches (BP) of the process are observed in close contact with the main process and these branches are devoid of organelles. 46,000x.
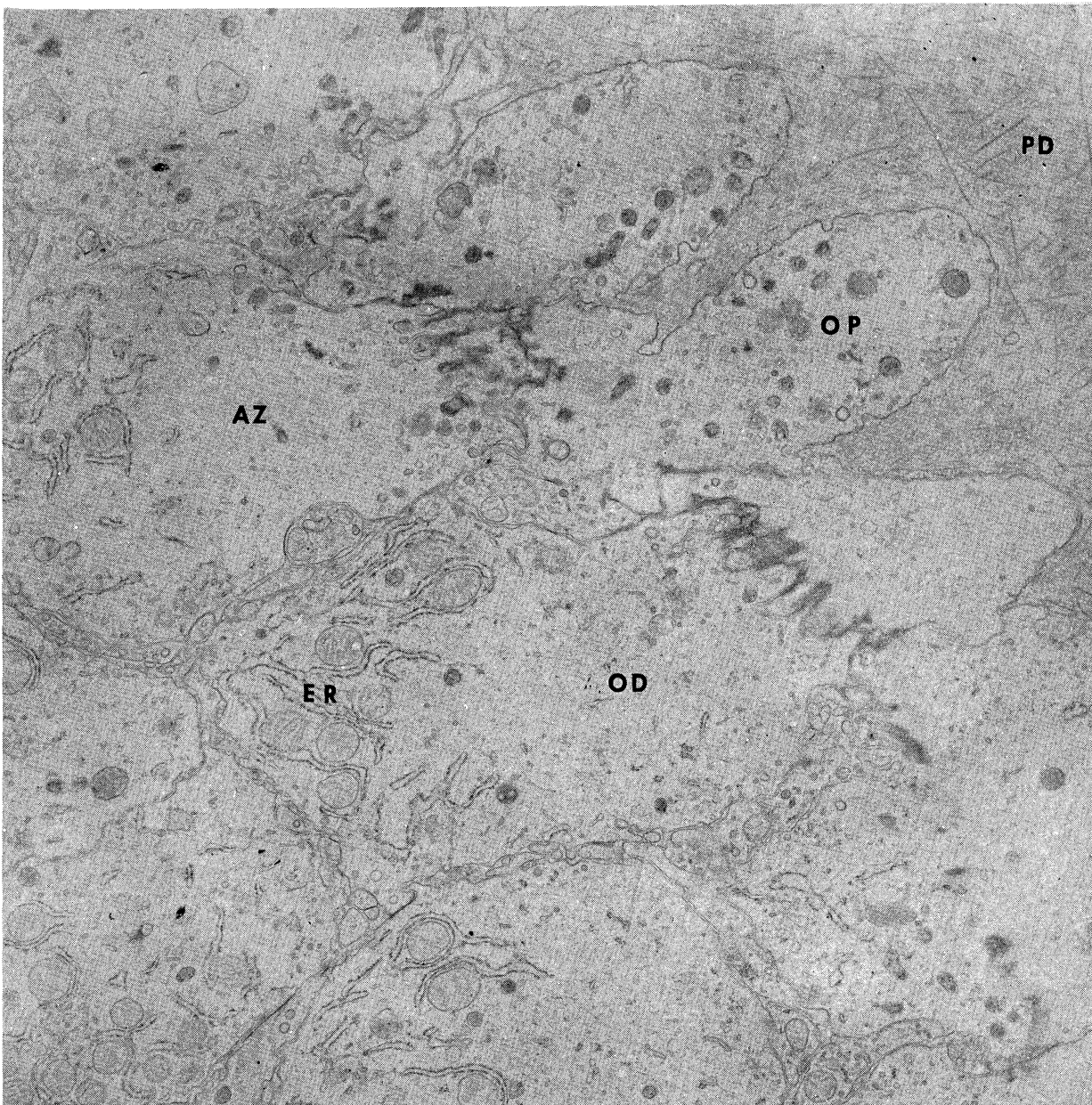
Fig. 16. A low power magnification of the apical ends of several odontoblasts
(OD) from which the odontoblastic processes (OP) extend out into the predentin
(PD). The tissue is from a six day old mouse and illustrates the transition
of the morphology of the odontoblast from the process filled with granules and
and vesicles to the apical zone (AZ) of the cell which is relatively free of
organelles. The apical zone gradually blends into a cell proper where numer-
ous mitochondria and profiles of endoplasmic reticulum are observed. 14,000x.
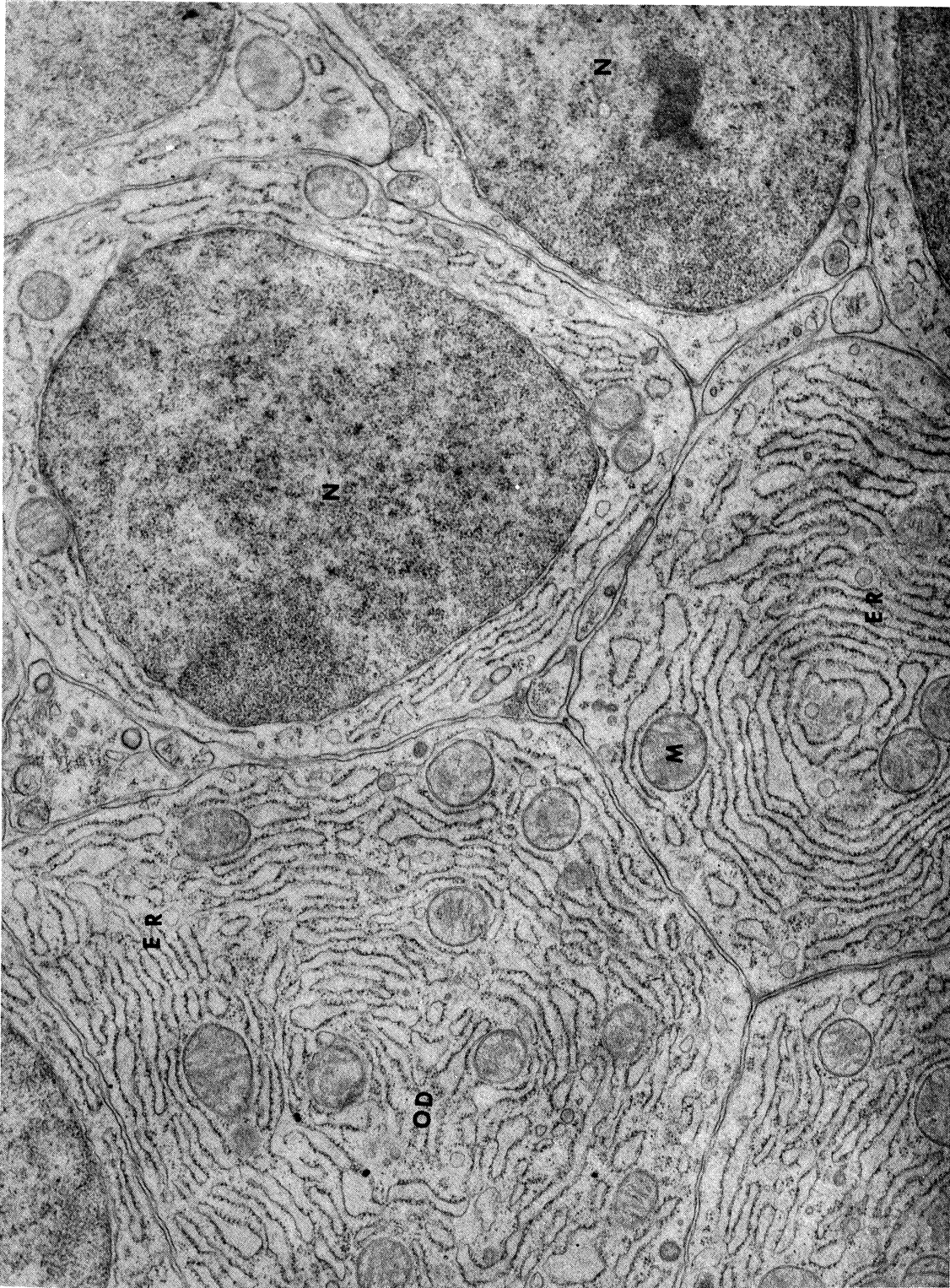
Fig. 17. A section through the nuclear level of several odontoblasts (OD) of a seven day old tooth. The rough-surfaced endoplasmic reticulum (ER) is well organized and is located both basally and surrounding the nucleus (N). Mitochondria are interspersed among the cisternae of the endoplasmic reticulum (ER). 36,400x.
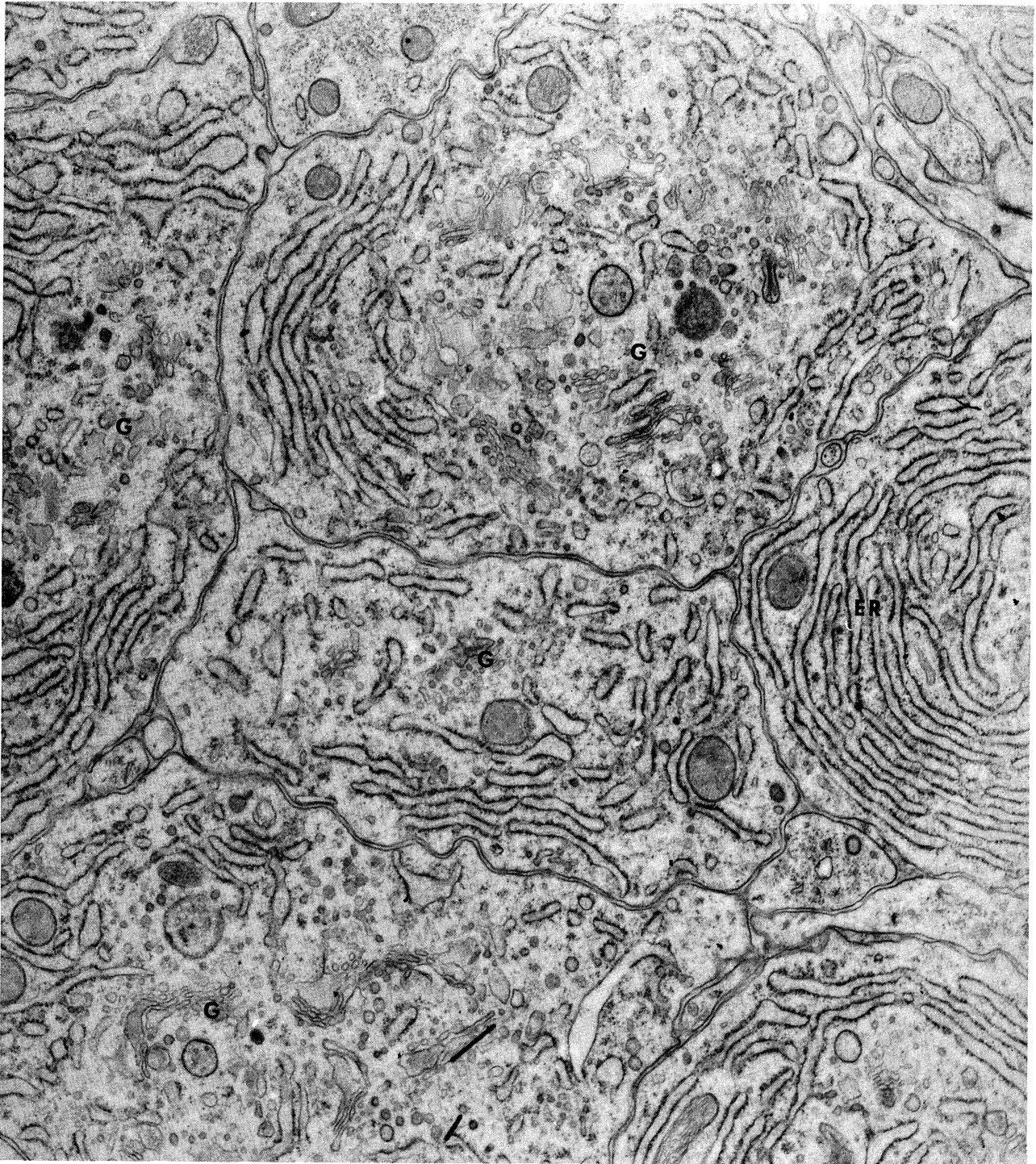
Fig. 18. A section through the Golgi apparatus (G) of several odontoblasts of a one day old mouse molar. The Golgi components include numerous Golgi vesicles, elongated flattened profiles, and dilated vacuoles. Rough-surface endoplasmic reticulum surround the Golgi apparatus. 29,800x.
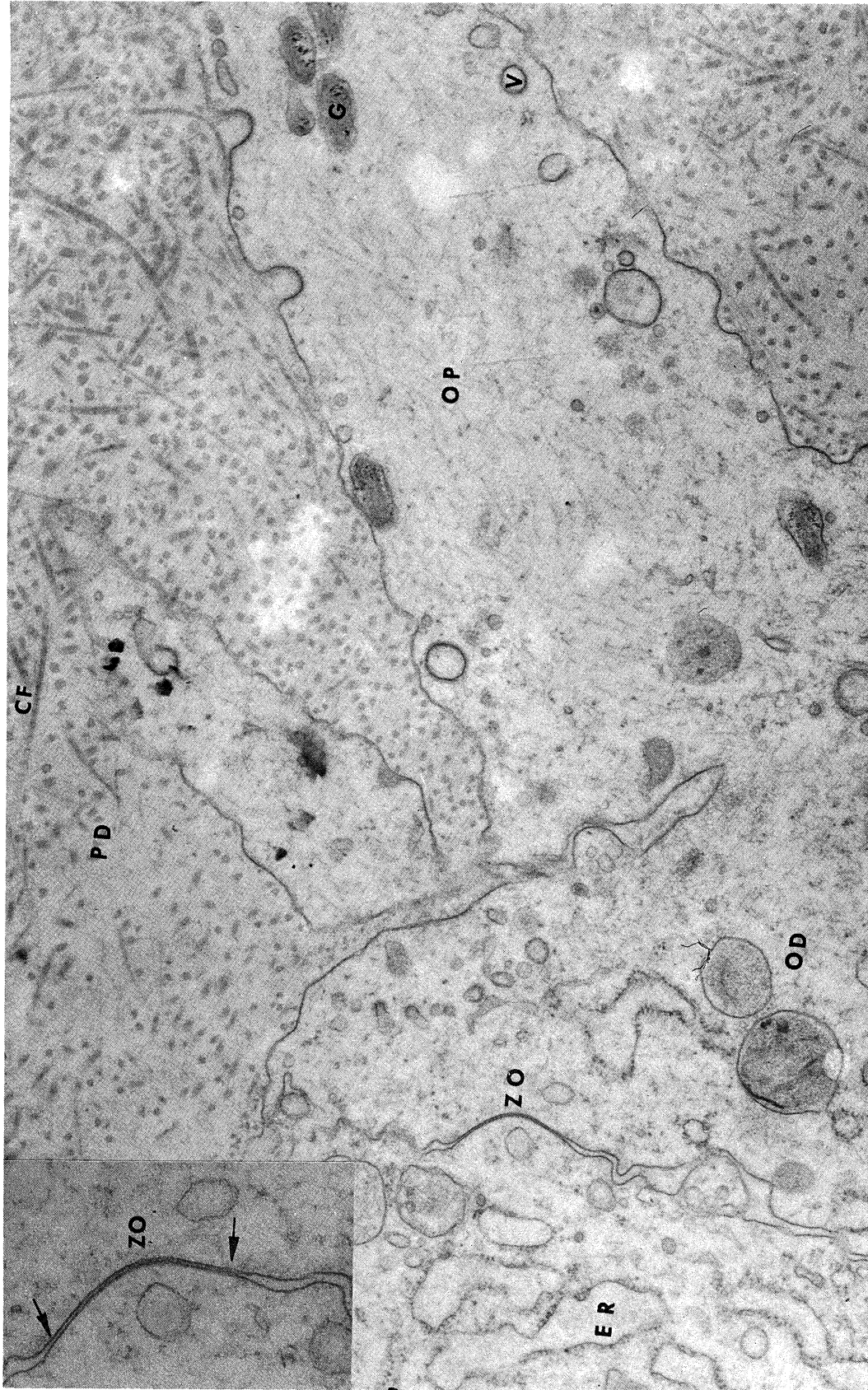
Fig. 19 A view of the apex of an odontoblast (OD) with two processes (OP) extending into the predentin (PD). The section is from a four day old tooth and demonstrates a Zona occludens (ZO) or tight junction between two adjacent odontoblasts. Granules (G) and vesicles (V) are located in the odontoblastic process and the apex of the odontoblasts. Rough-surfaced endoplasmic reticulum is observed at the apex of one odontoblast. 36,200x.
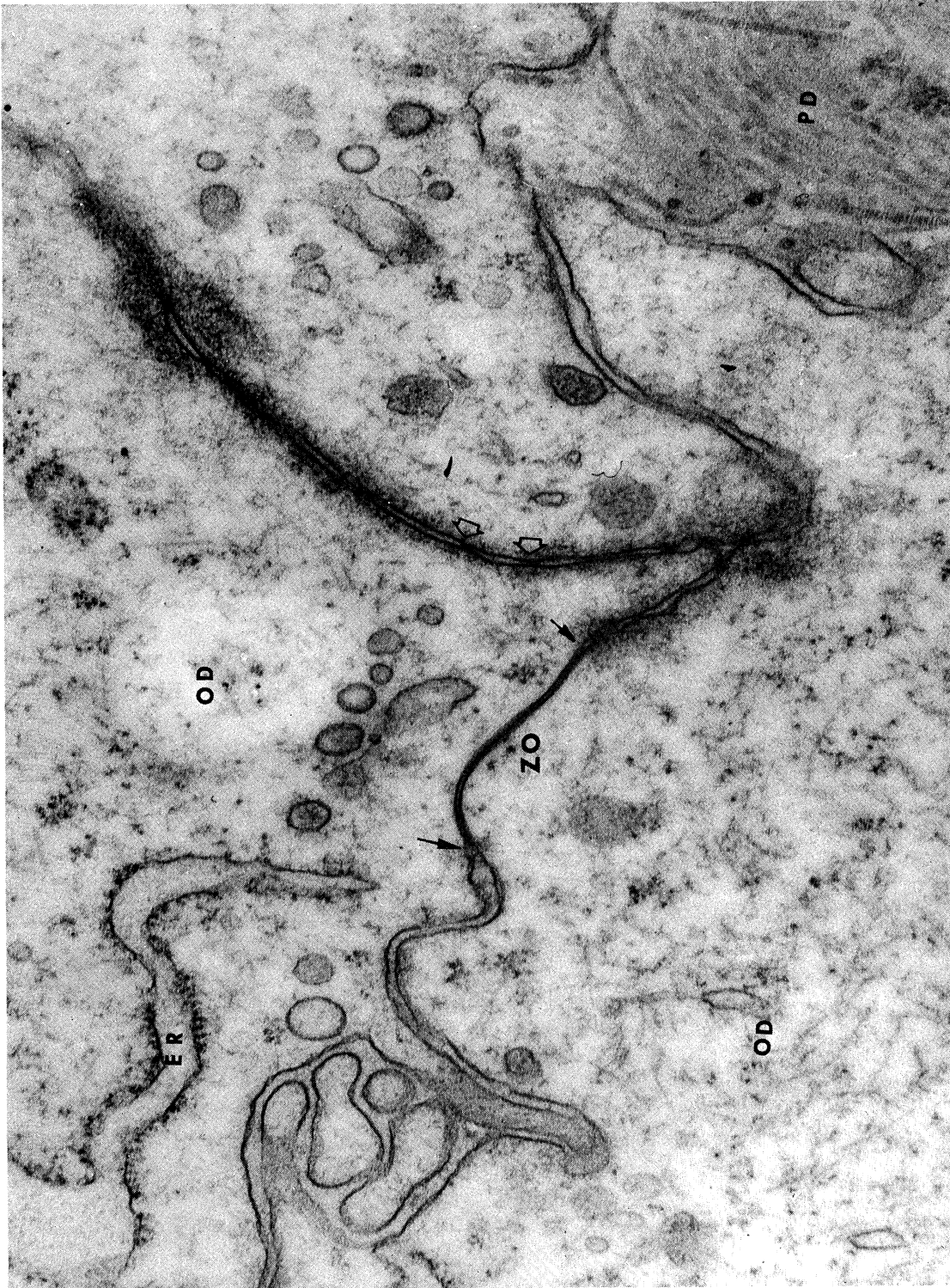Insert - a higher magnification of the Zona occludens (ZO). 68,000x.

Fig. 20 An area of cellular junctions of several odontoblasts (OD) in which a tight junction (ZO) is located between the solid arrows. The right branch of the V-shaped membrane structure illustrates several areas of contact of the adjacent membranes (outline arrows) and may represent a developing tight junction. 62,000x.
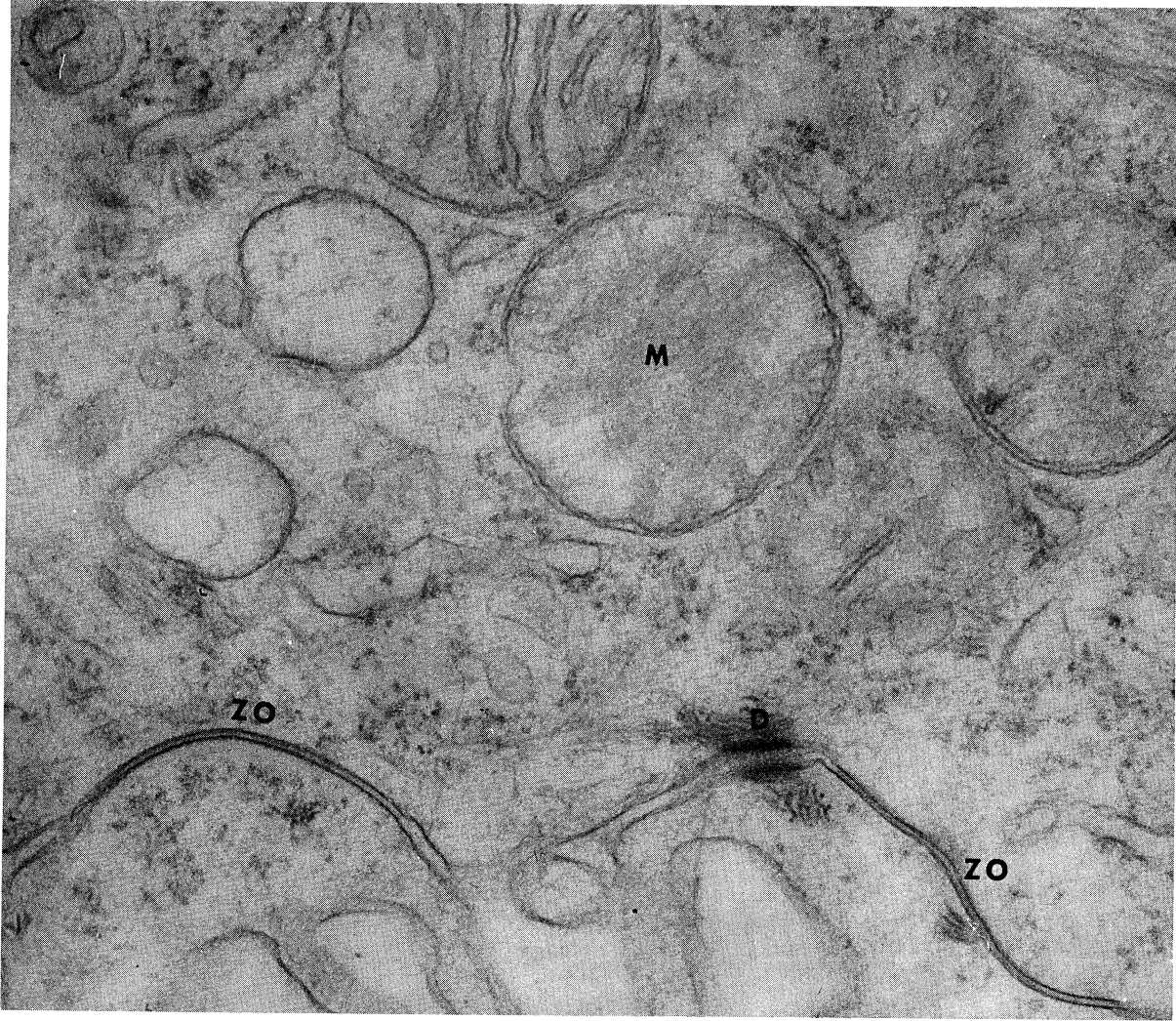
Fig. 21. An area of intercellular junctions between two odontoblasts of an
11 day old tooth in which two tight junctions (ZO) and a desmosome
(D) are observed. Permanganate stained. 81,000x.