The structure and distribution of nerves in the pulp-dentin organ

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The nerves in the pulp and the adjacent predentin and dentin in human teeth have been studied with light and electron microscopy. Myelinated and non-myelinated nerve fibers were found in the pulp as far peripherally as the cell-free zone. Difficulties were encountered in the demonstration of nerve fibers in the odontoblast layer due to the lack of definite criteria for the distinction of nerve fibers among the great number of cell processes in this location. In the predentin and dentin distinct nerve like structures were usually located in close relationship to the odontoblast processes and in the periodontoblastic space.

Key-words: Dental pulp; nerve tissue; microscopy, electron

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While the histology of dental innervation has been subjected to rather extensive light microscopic investigations, the study of the ultrastructure of nerves in the pulp and dentin has been somewhat sparse (*Engström & Öhman*, 1960; *Frank*, 1966, 1968; *Cahen*, 1969; *Eifinger*, 1970). *Arwill* (1958) in an extensive survey of the literature, concluded that many of the light microscopic investigations by previous workers were of limited value for various reasons. One of the most important reservations included the risk of extrapolating findings from animals to human beings.

The main obstacle to the study of dental innervation of human teeth is the technical difficulties involved in the preparation of sections (*Engström & Öhman*, 1960). Fairly good preservation of structural details may be obtained by the use of sections from human pulps removed from split teeth (*Furseth & Mjör*, 1969). The present study was projected to obtain further anatomical information concerning nerves in the pulp-dentin organ by aid of light and electron microscopy. Particular consideration was focused on regional variations in this innervation.

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MATERIAL AND METHODS

Twenty premolars from children aged 10—13 years were immediately cleaved (*Furseth & Mjör*, 1969), and the pulps were removed and placed in a 2.5 per cent glutaraldehyde solution buffered at neutral pH. Small pieces of predentin and dentin sometimes remained attached to the pulps. After fixation, pieces from the different regions of the pulp were isolated and post-

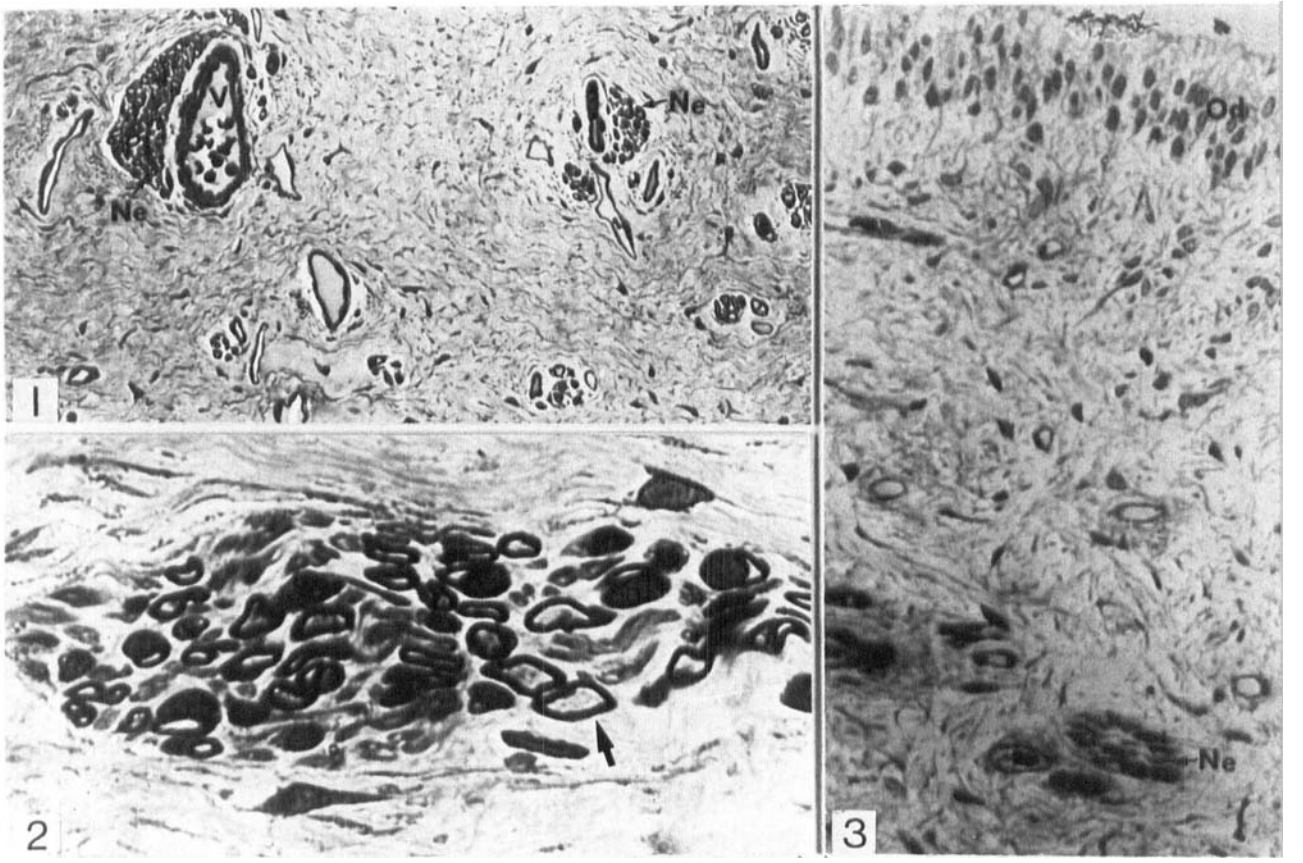


Fig. 1. Light micrograph from the central area of the pulp with several nerves (Ne) of varying sizes. Note their close relationship to the blood vessels (V). × 310
Fig. 2. Note the osmophilia of the myelinated fibers (arrow). Unmyelinated fibers are not identified at this magnification. No well defined perineurium can be distinguished. × 1200
Fig. 3. Light micrograph of the peripheral part of the pulp with the odontoblast layer (Od) to the left. Ne = myelinated nerve fibers. × 310

fixed in neutral buffered 1 per cent osmium tetroxide at 4°C for 2 hr (*Millonig*, 1961). After fixation, the blocks were rapidly dehydrated in a graded series of acetone and embedded in Vestopal W (*Ryter & Kellenberger*, 1958). Ultrathin sections were cut on an LKB Ultrotome III. The sections were examined in a Siemens Elmiskop Ia electron microscope. From the same plastic blocks, sections 1 μm were prepared for light microscopy. These sections were stained on a heating stage with an aqueous solution of 0.1 per cent toluidine blue adjusted to pH 8.9 with 0.67 M Na_2HPO_4.

OBSERVATIONS

Light microscopy

Numerous nerve branches of varying sizes can easily be identified in the 1 μm thick toluidine blue stained sections from the central area of the pulp, due to the osmophilia of the myelin (Figs. 1—2). They are usually located in the vicinity

Fig. 4. Electron micrograph illustrating details of a nerve from the central area of the pulp with myelinated (Al) and unmyelinated nerve-fibers (A). All fibers are invested by Schwann cell cytoplasm (arrows). N = nucleus of a Schwann cell. × 12000
Fig. 5. From the cell-free zone where only unmyelinated nerve fibers (Ne) are encountered. N = nucleus of Schwann cell. × 24000

of the blood vessels. The nerves which seem to be deprived of a distinct perineurium are composed of myelinated and unmyelinated fibers of varying sizes (Figs. 1—2). From these central branches, smaller bundles arborize towards the peripheral part of the pulp (Fig. 3). Myelinated fibres are not seen farther peripheral than the cell-free zone of the pulp, nor are any specialized forms of nerve endings identified.

Electron microscopy

Central pulp. The centrally located nerves are composed of numerous myelinated and unmyelinated fibres of different sizes. All the fibres are characterized by their investment of Schwann cell cytoplasm (Fig. 4). The nerves are seen adjacent to different kinds of blood vessels, but membranous contacts between the nerve fibres and the smooth muscle cells are not observed. In the more peripheral part of the pulp, the myelinated fibres are less frequent, but numerous non-myelinated bundles of axons of varying sizes are seen. Specialized nerve endings are not observed.

The cell-free-zone. Only unmyelinated fibres are encountered in the cell-free zone (Fig. 5). There seems to be a rather pro-

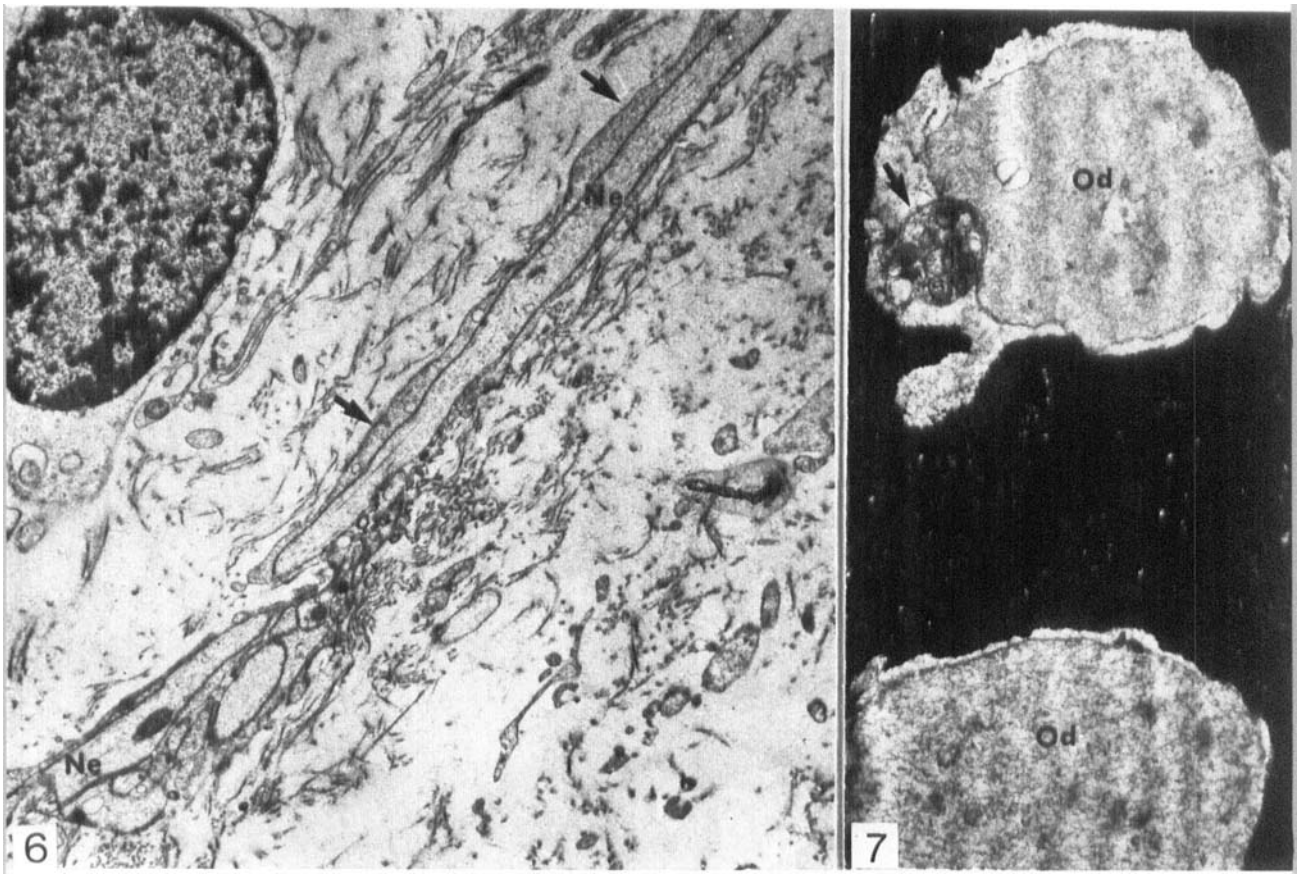


Fig. 6. Single nerve fiber (Ne) enfolded in a Schwann cell cytoplasmic sheath (arrows) is seen adjacent to the odonto blast layer. N = nucleus of an odontoblast. × 12000
Fig. 7. In the pulpal dentin, cytoplasmic processes containing several mitochondria, and with the appearance of nerve terminals (arrow) are seen in the periodontoblastic space. Od = odontoblast processes. × 18000

fuse ramification of the unmyelinated nerve bundles in this area, and in the vicinity of the odontoblast layer single nerve fibres, enfolded in a Schwann cell cytoplasmic sheath, may be observed (Fig. 6). Numerous neurofilaments are regularly seen in all the nerve fibres encountend in this area.

The odontoblast layer. While the different cell types with their cytoplasmic processes and nerve fibres are easily recognized in the more central part of the pulp (Figs. 4—6), several cytoplasmic processes encountered in the odontoblast layer are not as simple to identify. The odontoblasts are easily recognized by their rich content of cytoplasmic organelles, well developed Golgi zone in the juxta-nucleolar region, numerous mitochondria and rich amount of rough endoplasmic reticulum (Fig. 8). However, several scattered cytoplasmic processes of different appearance are also regularly encountered. Some of these contain small mitochondria and vesicles (Figs. 9, 11), or they are enfolded by cell cytoplasm, similar to the Schwann cell cytoplasm, while others are found in membranous contacts, as tight junctions, with

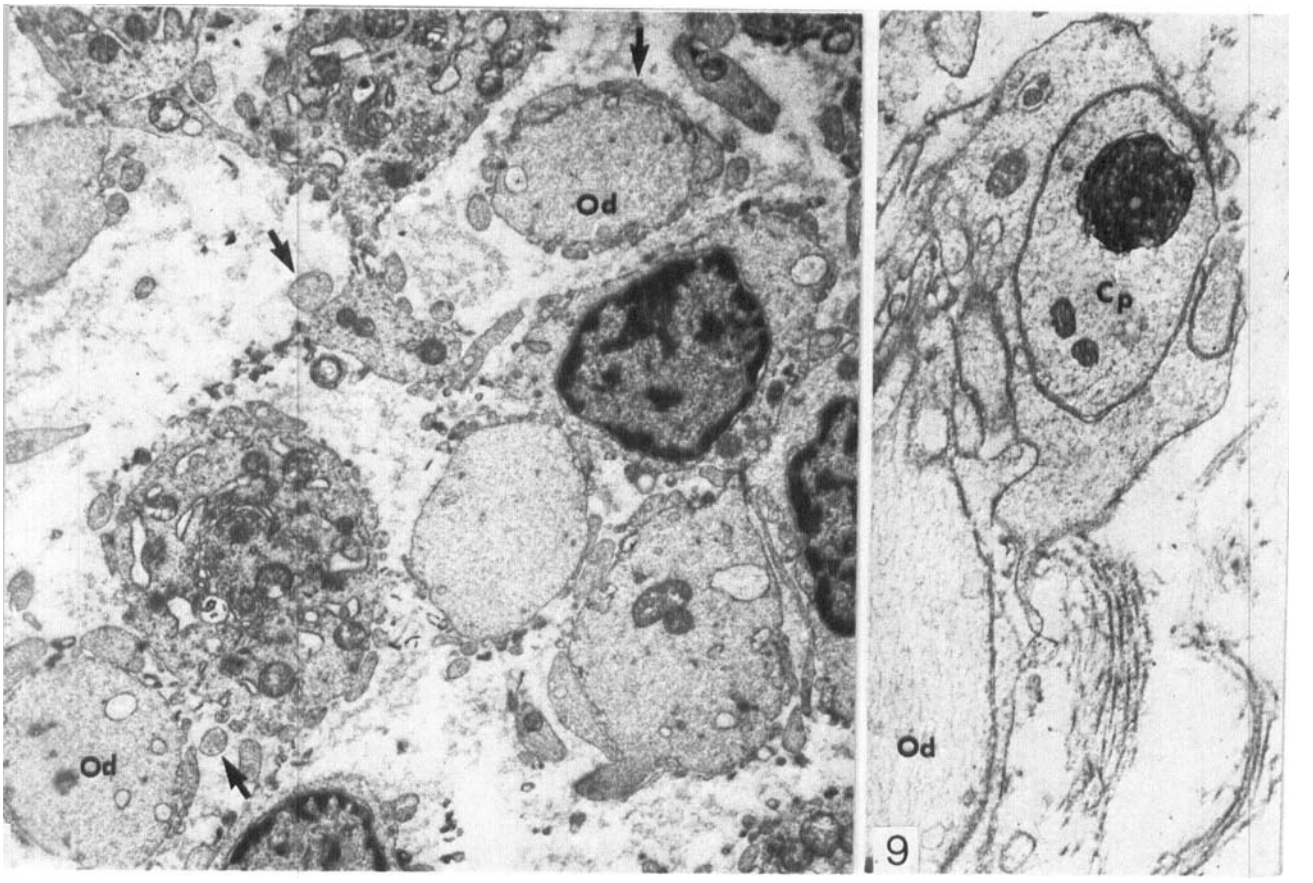
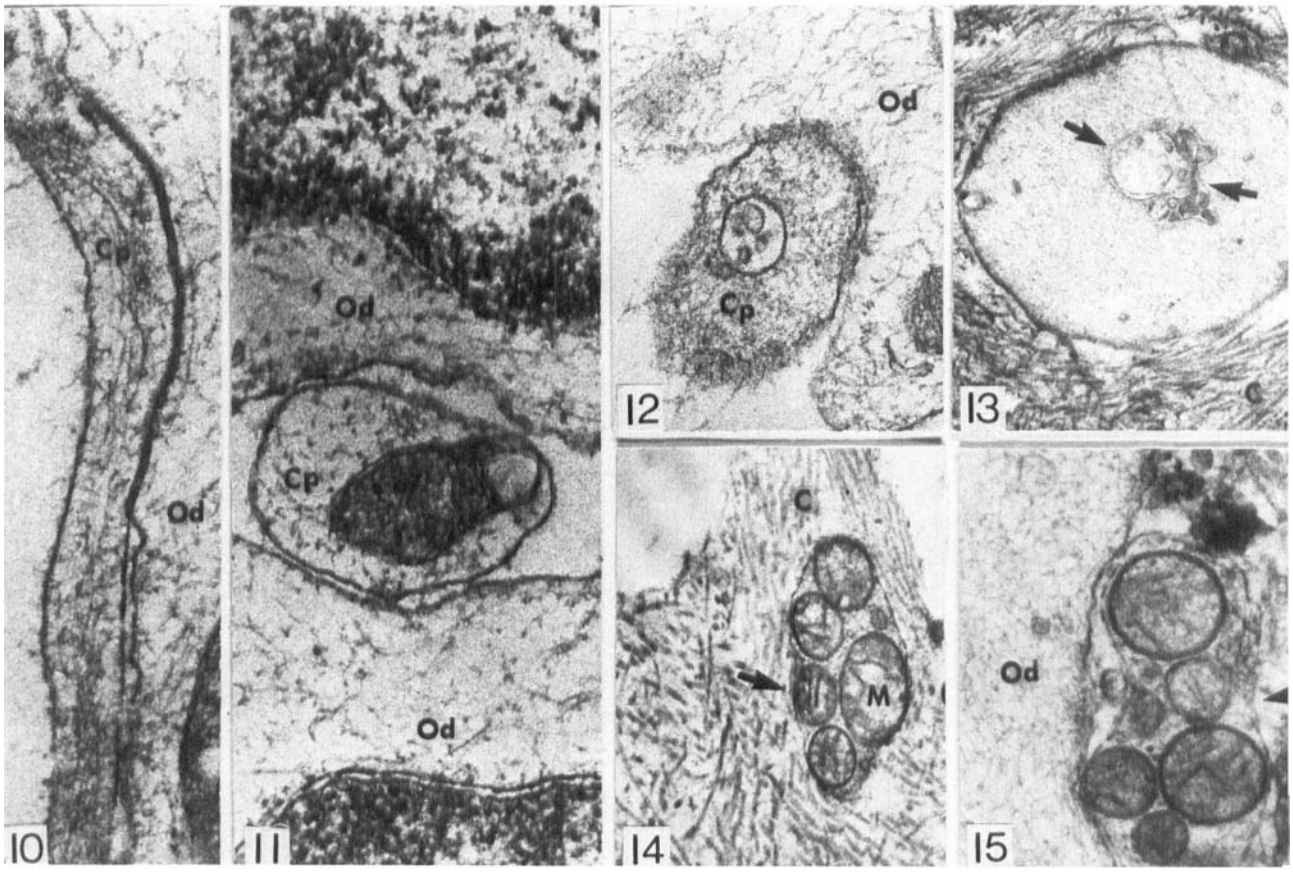


Fig. 8. Electron micrograph from the odontoblast layer with several odntoblast processes (Od) and numerous cytoplasmic processes (arrows) with variable morphology. × 12000
Fig. 9. Detail from the odontoblast layer with a typical odontoblast process (Od) and a cytoplasmic process (Cp) enfolded by cell cytoplasm similar to Schwann cell cytoplasm, suggesting it to be a nerve fiber. × 24000

odontoblast processes (Fig. 10). Lack of typical neurofilaments seems to be a striking feature of all the processes observed in this area.

The predentin — dentin area. In the predentin as well as in the pulpal dentin, cytoplasmic processes containing several mitochondria, and with the appearance of nerve terminals are occasionally observed (Fig. 7). A striking feature of these intradentinal extensions is that the cytoplasm is rather electron dense, in contrast to the pale odontoblast processes. The frequency of these cytoplasmic processes has not be determined, but they seem to be scant, and quite variable from one tooth to another and even from one area to another within the same tooth. They also seem to follow a fairly straight course along the odontoblast processes and are in contact with them (Figs. 10—12), located in the periodontoblastic space in a small recess in the odontoblast process (Fig. 7).

Periodontoblastic spaces are rarely observed in the predentin, while a space between the odontoblast processes and the mineralized wall of the dentinal tubules



Figs. 10—12 demonstrate different types of cytoplasmic processes (Cp) in membranous contact as tight junctions (Fig. 10) with odontoblast (Od) or only as close contact (Fig. 11). While some of the processes contain a network of small filaments (Figs. 10, 11) and are morphologically similar to odontoblast processes, others have an appearance coresponding more with a nerve fibre (Fig. 12). × 60000.
Fig. 13. Odontoblast process in the predentin containing intracytoplasmic membrane-limited structures (arrows). C=collagen matrix. × 14000
Fig. 14. Cytoplasmic process (arrow) with many mitochondria (M) located within the predentin. C=collagen matrix. × 24000
Fig. 15. Cytoplasmic process (arrow) located adjacent to odontoblast process (Od) in the predentin C=collagen matrix. × 40000

are regularly encountered, often also with lateral branches of the dentinal tubules. Characteristically, all the odontoblast processes in this area are relatively free of organelles. However, in some of the cytoplasmic processes, intracytoplasmic membrane-bound structures are encountered in the predentin area (Fig. 13). They are located rather centrally within the processes, and have an appearance similar to microbodies. Endoplasmic reticulum or ribosomes are not observed in relation to the structures described. Cytoplasmic processes containing many mitochondria can also be demonstrated within the predentin collagen matrix (Fig. 14), i.e. lacking the close relationship to odontoblast processes usually encountered (Fig. 15).

DISCUSSION

The classical silved impregnation technique of light microscopy demonstrated

numerous nerves in the pulp-dentin organ (e.g. *Arwill*, 1963; *Fearnhead*, 1963). Silver stains are notoriously capricious, however, and the possibility of confusing reticular fibres and small axons is ever present. With improvements in fixation and embedding methods for electron microscopy, it has been possible to obtain detailed information about the fine structure of the dental pulp and its nerve fibers (*Engström & Öhman*, 1960; *Arwill*, 1968; *Frank*, 1968; *Harris*, 1968; *Cahen*, 1969; *Eifinger*, 1970). The present study has confirmed previous light and electron microscopic observations that the dental pulp of the human is found to be profusely innervated. In the central part, the pulp was pervaded by nerve bundles composed of two distinct types of fibers, viz myelinated and unmyelinated. As the bundles of nerve fibers arborized towards the more peripheral part of the pulp, the myelinated fibers were less frequent, but numerous non-myelinated axons were seen, all characterized by their investment of Schwann cell cytoplasm. In the cell-free zone, only unmyelinated fibers were seen. The electron micrographs demonstrated this dental pulp innervation to be rather complex, and it is still not yet possible to identify all the observed structures with certainly or to estimate their functional significance. It should also be noted that the present material comprised young teeth and a different distribution may be found in teeth with completed root formation (*Fearnhead*, 1963).

In the odontoblast layer, cytoplasmic processes similar to nerve fibers were seen. It is conceivable that several of these processes may be nerve fibers, since no terminal nerve endings were found in the cell-free zone. On the contrary, single nerve fibers approaching the odontoblast layer were seen in this area. The identification of the cytoplasmic processes containing closely packed mitochondria which were accompanying the odontoblast processes in the dentinal tubules, is considered to be less speculative. Comparing these structures with those in previous reports (*Fearnhead*, 1961; *Frank*, 1966, 1968; *Arwill*, 1968), they most likely represent nerve fibers. Moreover, consistent with the reports of *Frank* (1968) and *Arwill* (1968) it has been demonstrated that the cytoplasmic processes accepted as nerve terminals in the dentinal tubules, were in membranous contacts with the odontoblast processes. Similar membranous contacts were not observed between nerve fibers and other cells or cytoplasmic processes in the pulp. The nerves were usually located in the periodontoblastic space, as demonstrated by other investigators, but nerve-like processes not closely related to odontoblast processes were also observed within the predentin. Since nerve fibers have to come from intercellular spaces, they must either run along the distal end of the odontoblast to the process or cross the predentin matrix before joining the process. The latter situation could explain the occasional lack of a close relationship.

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