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Periodontal neural endings intimately relate to epithelial rests of Malassez in humans
A light and electron microscope study

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ABSTRACT

The periodontal ligament was examined by light microscopy at 3 different levels (apical, intermediate, coronal) on the 4 root sides of 43 extracted teeth. Epithelial rests were localised and serial LM and EM sections showed a close apposition (up to 0.03 μm) between Ruffini-like and free nerve endings and the basal lamina of the epithelial cell rests. The neural structures were facing the epithelial cells, whereas the Schwann cells were oriented towards the outer connective tissue. The Ruffini-like corpuscles contained numerous mitochondria. Free nerve endings contained neurotubules, neurofilaments and some vesicles. The intimate association between both neuroectodermal structures could indicate a target function of epithelial cell rests during developmental periodontal ligament innervation. Recent immunohistological findings involving nerve growth factor receptors substantiate this hypothesis.

INTRODUCTION

A specific cell population in the developing and established periodontal ligament is derived from the disintegrating sheath of Hertwig during tooth root development, namely, the epithelial rests of Malassez (ERM) (Malassez, 1884, 1885*a*, *b*). Their morphology and distribution have since been studied by many investigators (e.g. Ten Cate, 1965; Valderhaug & Nylen, 1966). Only few reports have described their ultrastructure (Hamamoto et al. 1989; Yamasaki & Pinero, 1989). An in vitro study indicated that these epithelial cells can migrate and have a propensity for differentiation (Yamasaki & Pinero, 1989). In vivo they can be activated on stimulation (Johansen, 1970). The reason for their persistence for years after completion of permanent dentition is still unknown. They might be functioning in cement apposition and resorption and prevention of ankylosis (Loë & Waerhaug, 1961). They have been observed to be associated with periodontal cyst formation (Ten Cate, 1972) and apical migration of the pocket epithelium (Spoughe, 1984). Following some chance ultrastructural observations of a close apposition of nerve endings with ERM, the present study investigated systematically the possible interrelationship between ERM and periodontal nerve endings.

MATERIAL AND METHODS

Forty-three teeth with a healthy periodontium (8 molars, 13 premolars, 5 canines and 17 incisors) were extracted for prosthetic or orthodontic reasons. The mean age of the patients was 37.2 y (S.D. 14.7) (6 aged 10–20 y, 7 at 21–30, 4 at 31–40, 2 at 41–50, 11 at 51–60, 1 between 70 and 80 y). The teeth were fixed with a solution of 2% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.3) for 24 h. Pieces of tooth-related periodontal ligament were dissected under a stereomicroscope and classified according to their position in the periodontal ligament (apical, intermediate, coronal). The pieces were further fixed by immersion for 4 h in the same solution. The small pieces were washed in 0.05 M cacodylate buffer for 10 min, postfixed in 2% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.3) for 1 h, washed, dehydrated through graded concentrations of acetone

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and propylene oxide and embedded in Araldite. Semithin sections were cut on a Reichert Ultracut microtome perpendicular to the axis of the root. Thereafter the sections were stained with a solution of thionin-methylene blue (0.1% aqueous solution). Serial semithin sections ($\sim$ 0.5 μm) were examined by light microscopy. Serial ultrathin sections ($\sim$ 0.06 μm) were prepared every 100 μm and mounted on 0.7% formvar-coated grids, stained with uranyl acetate and lead citrate in an LKB Ultrostainer and finally examined with a Philips EM 400 electron microscope. A total of 496 sections out of 1050 could be used for the histological investigation. The others presented too much tissue disruption as a result of the extraction of the teeth.

RESULTS

Light microscopy

Epithelial cell rests of Malassez were systematically sought in the tooth-related part of the periodontal ligament of the extracted teeth. In total, 23 ERM were detected. They appeared at all levels with respectively 7, 7 and 9 in the coronal, intermediate and apical parts. On the serial section no interconnection between the islands could be detected. The existence of a network thus could not be visualised. Usually the cell rests appeared as oval or round clusters of cells in sections cut perpendicular to the root axis. In longitudinal sections the epithelial cells were arranged in strands. On serial semithin sections a close to intimate apposition (up to 0.5 μm) of neural structures to ERM was observed in 20 out of the 23 ERM. In general, the density of neural structures in the examined tooth-related parts of the periodontal ligament was low. Myelinated nerve fibres (average diameter 5 μm) lost their myelin sheaths in the vicinity of the ERM and reached them as unmyelinated preterminal axons (Figs 1, 2). These neural structures sometimes surrounded the epithelial cell rests.

Electron microscopy

The epithelial cells were easily recognised by their round to ovoid, partly invaginated nuclei. The nuclei had condensed heterochromatin and 1 or 2 nucleoli (Fig. 3). The cytoplasm of these cells contained the normal set of organelles, glycogen particles and bundles of microfilaments. Several hemidesmosomes were observed at the periphery of the cell rests. A prominent basal lamina was present between the cell membranes and the connective tissue. The epithelial cells were joined to each other by desmosomes and gap junctions. Serial EM sections showed neural structures in the immediate vicinity of the epithelial rests. The mean distance on 20 sections showing 46 associations between ERM and neural endings was 0.5 μm (S.D. 0.31 μm). The neural structures were either Ruffini-like receptors or free nerve endings. The former mostly showed neural expansions containing numerous mitochondria (Figs 3–7). Unmyelinated axons (Fig. 9) contained microtubules, neurofilaments and some clear-cored vesicles. The axons mostly faced the epithelial cells while the Schwann cells were usually located towards the outer connective tissue. A close apposition (up to 30 nm) between these neural structures and ERM was regularly encountered.

In some instances there were substantial numbers of pinocytotic vesicles in the surface membrane of the ensheathing Schwann cell (Figs 5, 6). These vesicles were oriented towards the outer connective tissue and the connective tissue between the nerve ending and the ERM. Vesicle density was greater on the inner side. In other instances neural endings which presented an intimate relationship with the ERM (Fig. 7*a*, *b*) also contained vesicles. At times the ERM was completely surrounded by neural structures (Fig. 8).

DISCUSSION

The function of the ERM is still a matter of debate. The frequent association (20 out of 23 ERM) of nerve endings and ERM, both of neuroectodermal origin, was very obvious in the present observations. The low incidence of ERM in the adult periodontal ligament is in accordance with earlier reports (Reeve & Wentz, 1962). A dramatic decrease in the number of ERM occurs after the second decade. However, in the present study the density was not significantly higher in the samples obtained from patients younger than 20 years. The high degree of association between neural and ectodermal structures contrasts sharply with the moderate density of the neural endings in the rest of the tooth-related part of the periodontal ligament. A plausible explanation for this association may be a

Fig. 1. (*a*) Light micrograph of the PDL of an extracted tooth showing a myelinated nerve fibre (★) in the vicinity of an epithelial cell rest of Malassez (*ERM*). (*b*) At a higher level (1 μm away) the nerve fibre loses its myelin sheath (▲). The nerve fibre lies against the ERM while the Schwann cell nucleus (*S*) faces the connective tissue. ×3600.

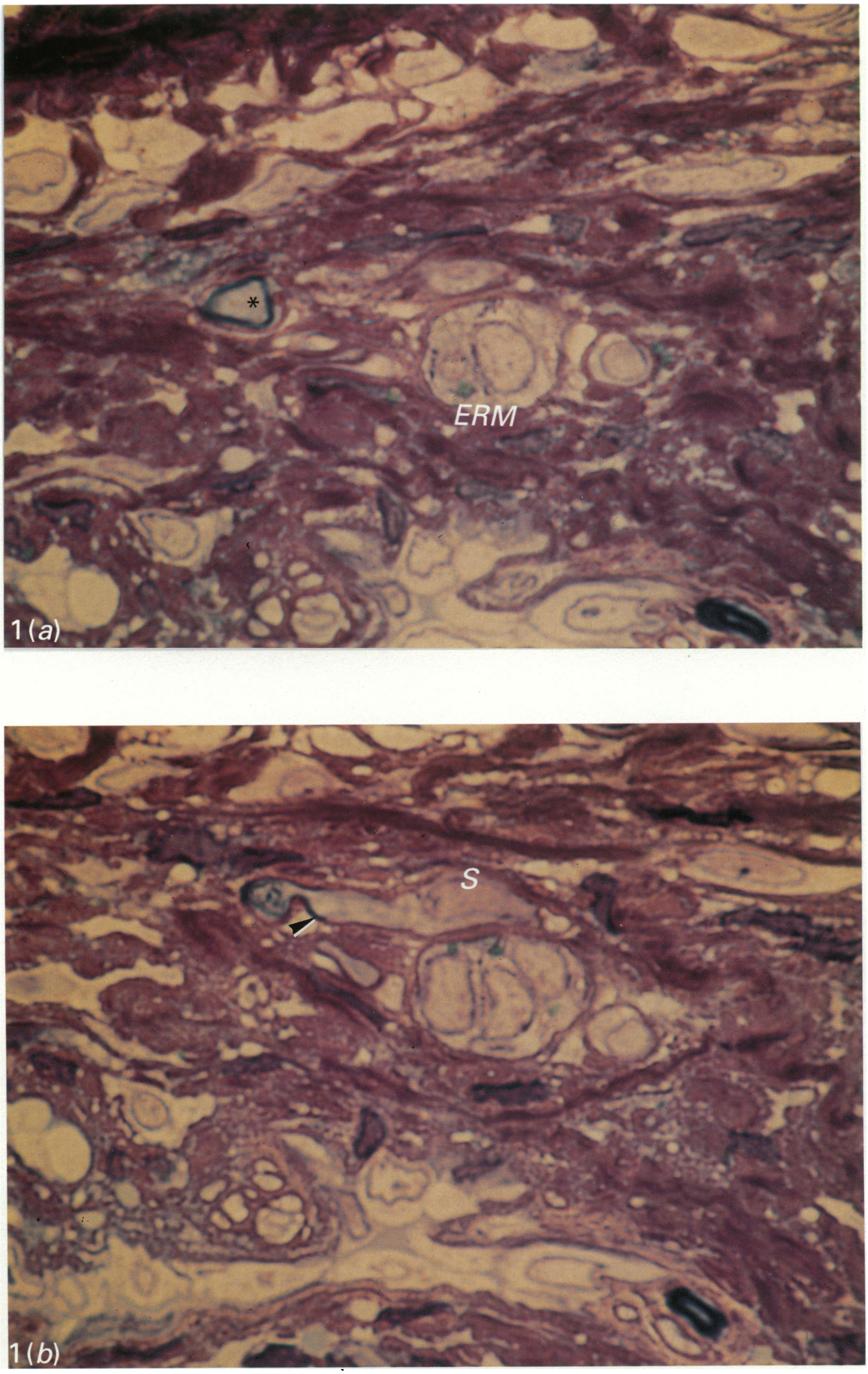

Fig. 1 (*a*, *b*). For legend see opposite.

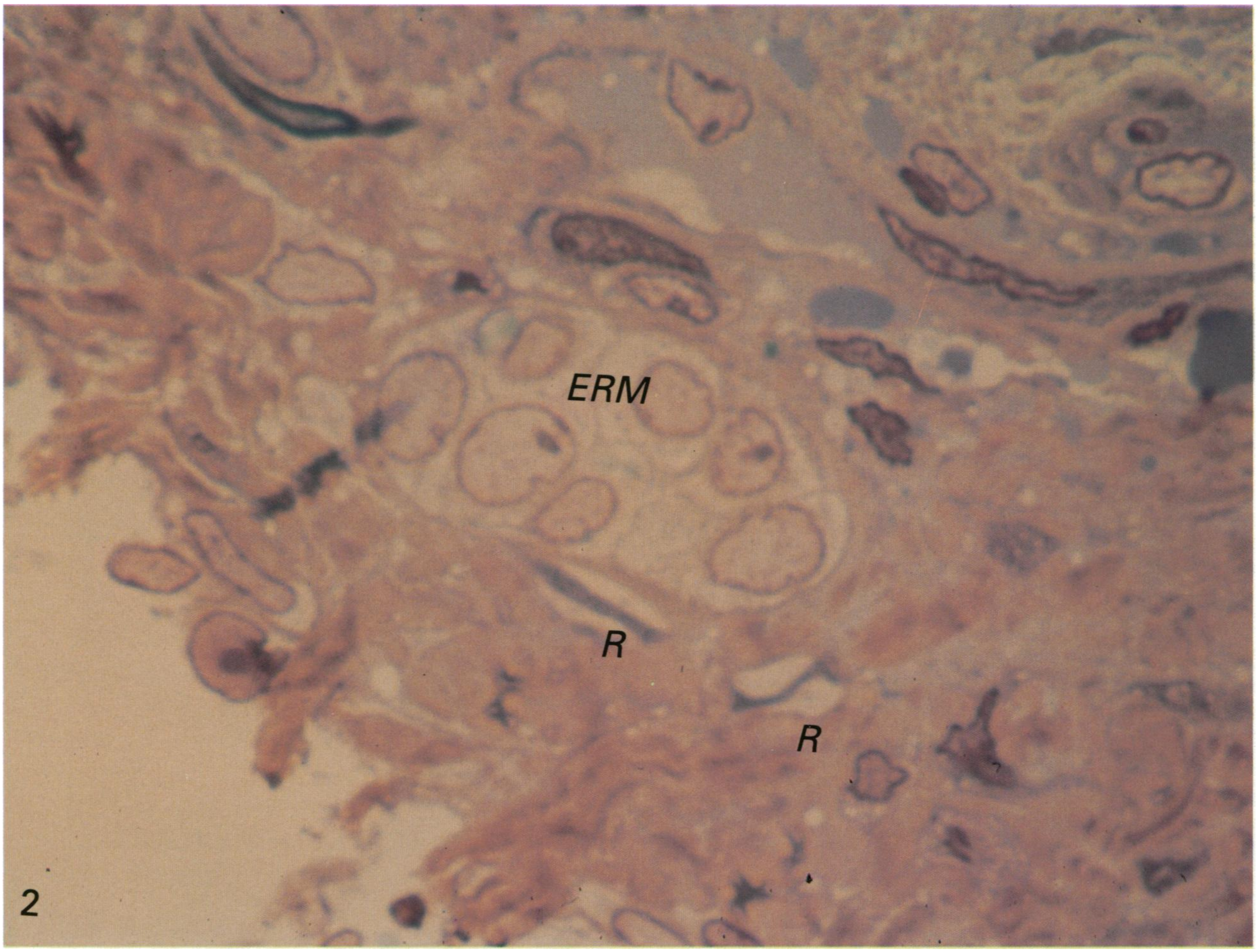

Fig. 2. Two Ruffini-like endings (*R*) located near the bottom of the *ERM*. The Ruffini-like endings can easily be identified in the EM section shown in Fig. 4, which is from the same ERM but at a different level. ×4000.

target function of the ERM for the growing nerve fibres. Similar associations between epithelial cells and nerve endings are well known elsewhere in the periodontium, namely the Merkel receptors (Merkel, 1880). Merkel cells, which are epithelial–neurite complexes, are present in the gingival epithelium (Hashimoto, 1972). They are considered to be transducers of mechanical stimuli to the nerve cell by means of secretory granules. Their location in the cell is polarised towards the nerve and they are part of a chemical transmitter system (Munger, 1977). Although it can be postulated that the neuroectodermal association functions more or less as a specialised receptor, it should also be considered as a targeting mechanism during nerve growth. During growth the budding nerve fibre seeks its target epithelial cell either by chemotropism (e.g. nerve growth factor, NGF) or random searching and recognition (English et al. 1980). For the Merkel cell it is known that the cell exists before the arrival of nerve fibres (Scott et al. 1981). The role of targeting and nerve growth inhibition is by contact with the basal lamina (English et al. 1980). NGF receptors have also been identified in the junctional epithelium of the gingiva, which is indicative of nerve growth regulatory mechanisms in nonneural tissues (Thompson et al. 1989). NGF receptors were also detected at the external root sheath of their hair follicles (Thompson et al. 1989).

A decisive argument in favour of the present hypothesis is the recent discovery of NGF receptors in the epithelial rests of the dental follicle of unerupted human third molars (Lambrichts, Creemers, van den Oord and van Steenberghe, unpublished observa-

Fig. 3. Epithelial cells (*ERM*) with invaginated nuclei (*N*) containing heterochromatin and nucleoli (*No*). Tonofilaments are sometimes associated with desmosomes (↑). Hemidesmosomes can also be seen (★). In close vicinity to the basal lamina, Ruffini-like neural endings (*R*) filled with mitochondria can be seen. A free nerve ending is also present (▲). ×12000.

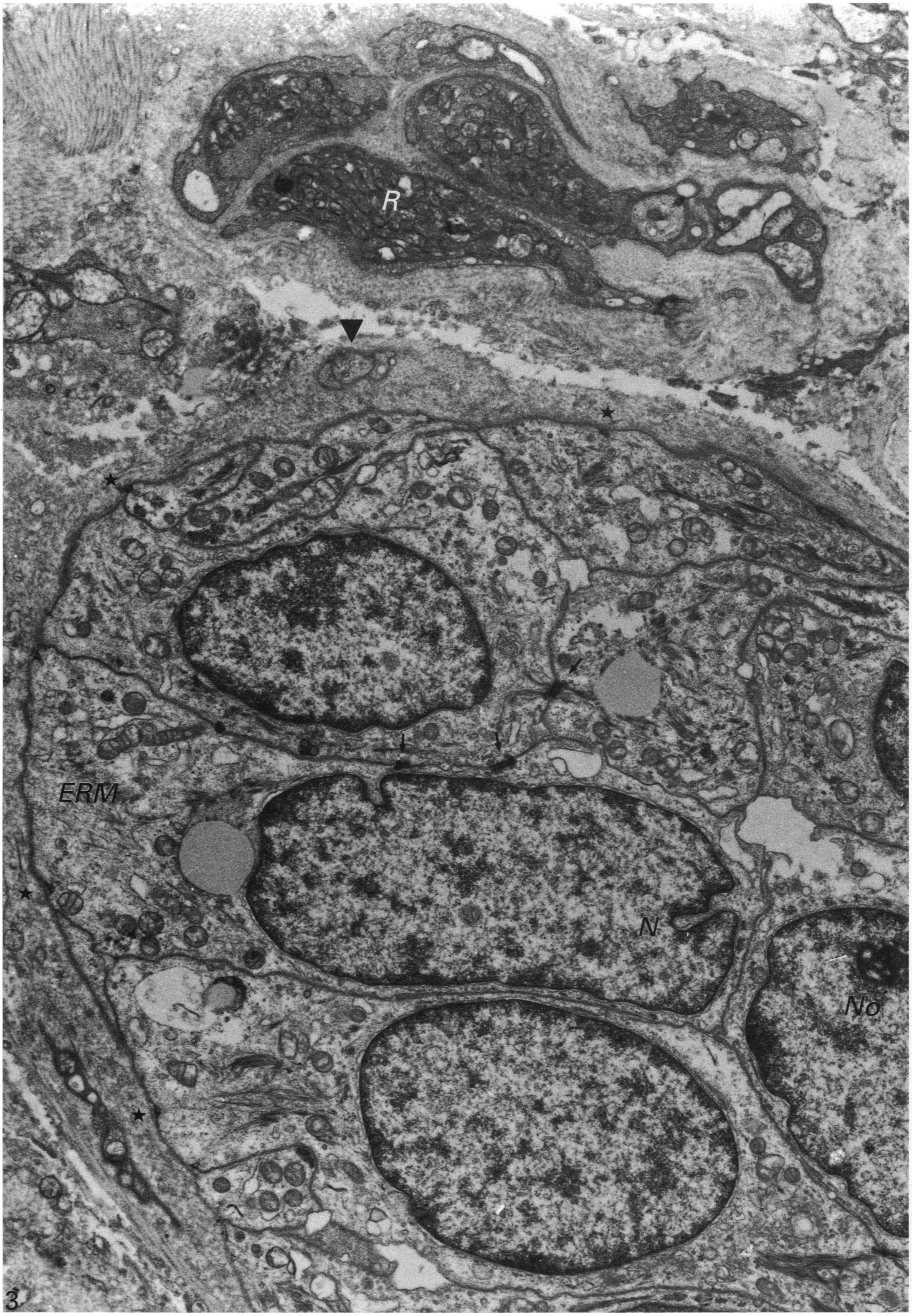

Fig. 3. For legend see opposite.

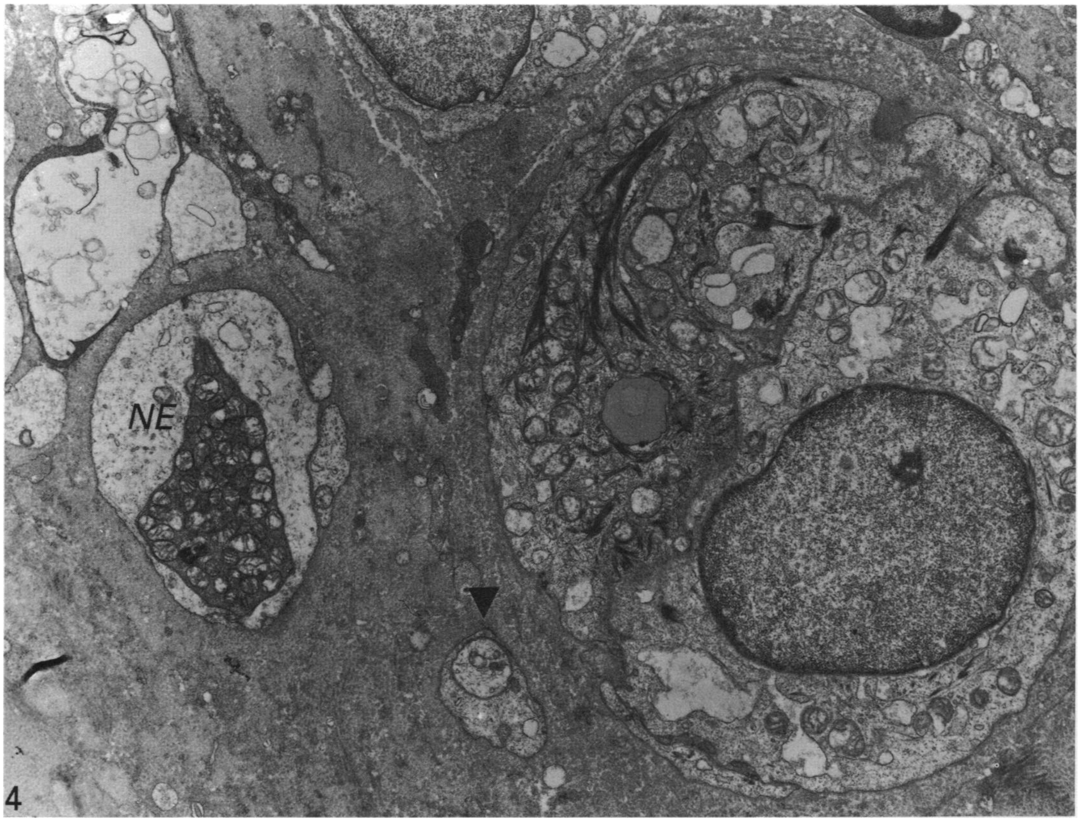

Fig. 4. Electron micrograph of the same ERM as in Figure 2 showing a typical close association between an ERM and a neural ending (*NE*). An unmyelinated axon is also present (▲). × 10450.

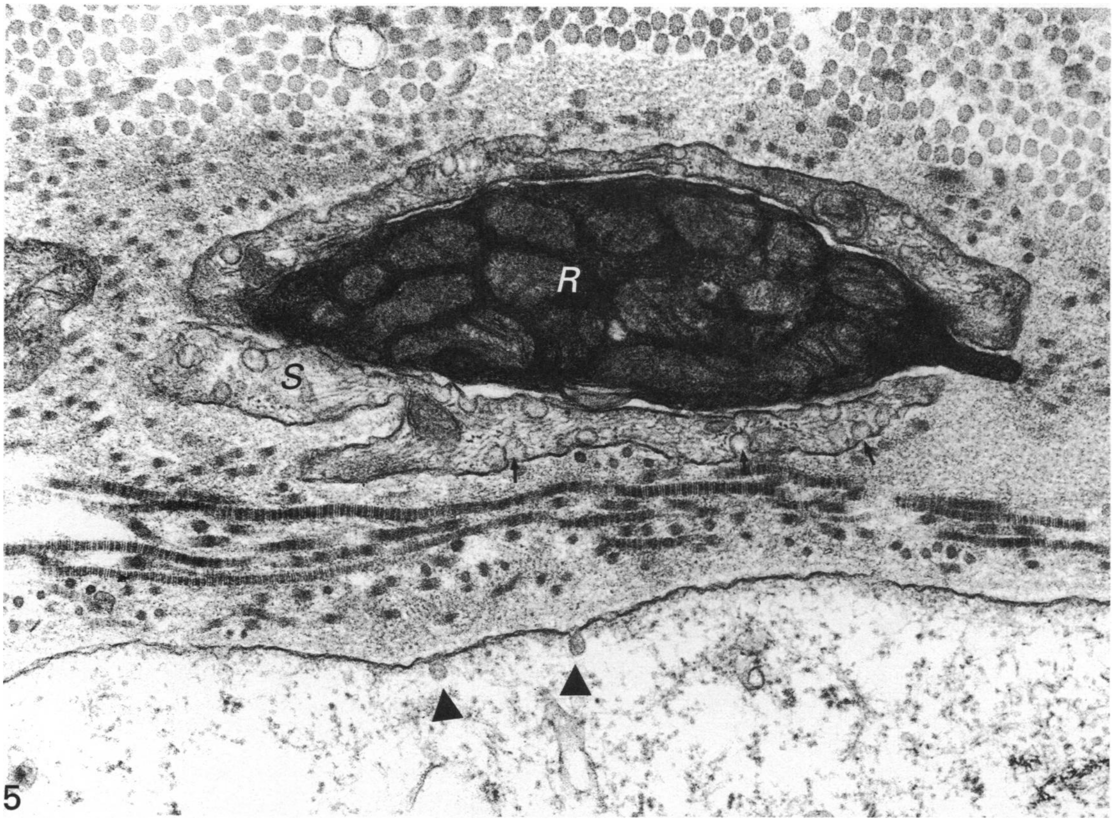

Fig. 5. Higher power electron micrograph of a Ruffini-like ending (*R*) surrounded by a Schwann cell (*S*) in the direct vicinity of an ERM. Prominent pinocytotic activity (↑) can be seen in the Schwann cell. Some pinocytotic vesicles (▲) are present at the epithelial cell membrane. × 47500.

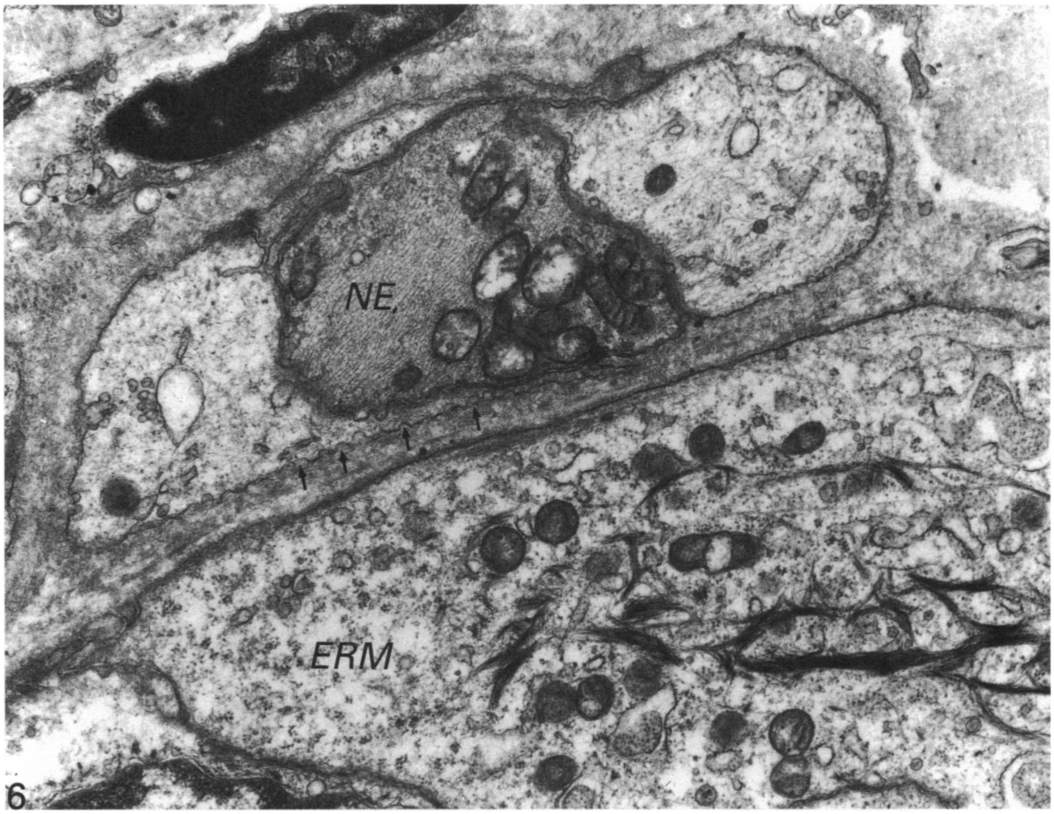

Fig. 6. Electron micrograph showing the same intimate interrelationship between a neural ending (*NE*) and an ERM as observed in Figure 5. Prominent pinocytotic activity (↑) can be seen in the Schwann cells facing the ERM. ×21150.

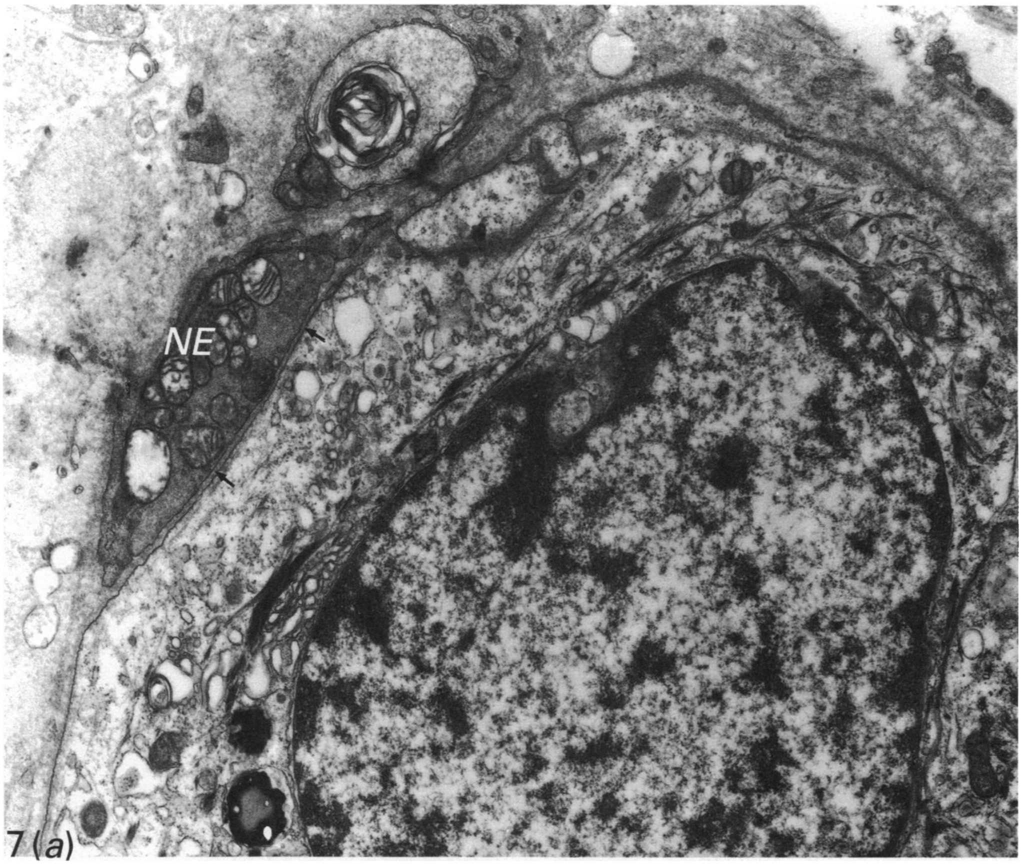

Fig. 7(*a*). For legend see p. 160.

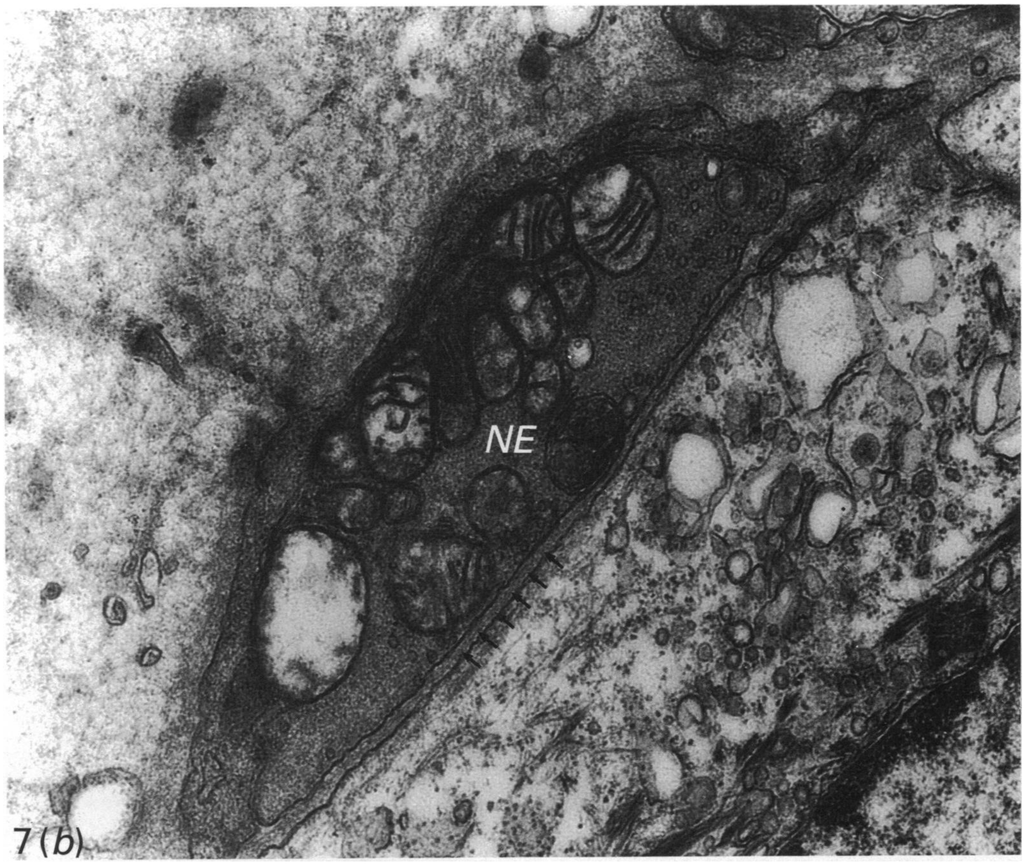

Fig. 7. (*a*) Electron micrograph showing the intimate association (30 nm) of an ERM and a neural ending (*NE*). There is a common basal lamina (↑). The neural ending contains mitochondria and vesicles. × 16200. (*b*) Higher power micrograph of the same ERM and a neural ending (*NE*). × 35100.

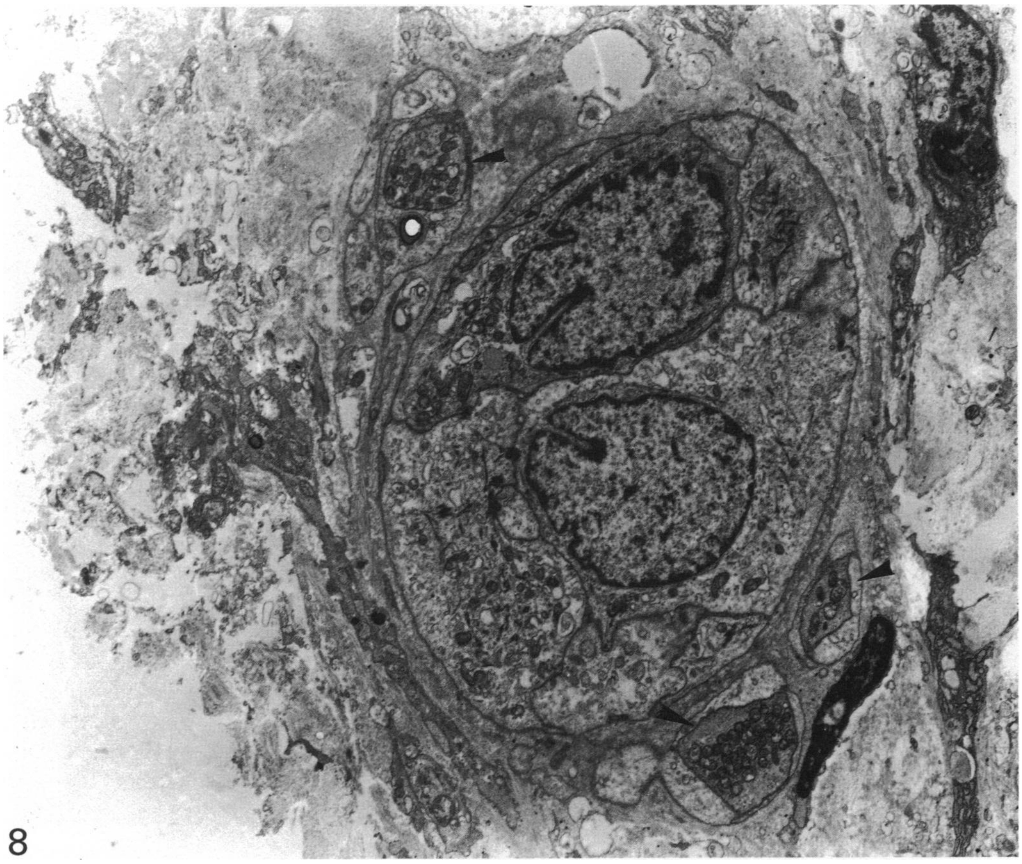

Fig. 8. An ERM is completely surrounded by a neural plexus. The neurites (▲) are of the Ruffini-like type. × 5940.

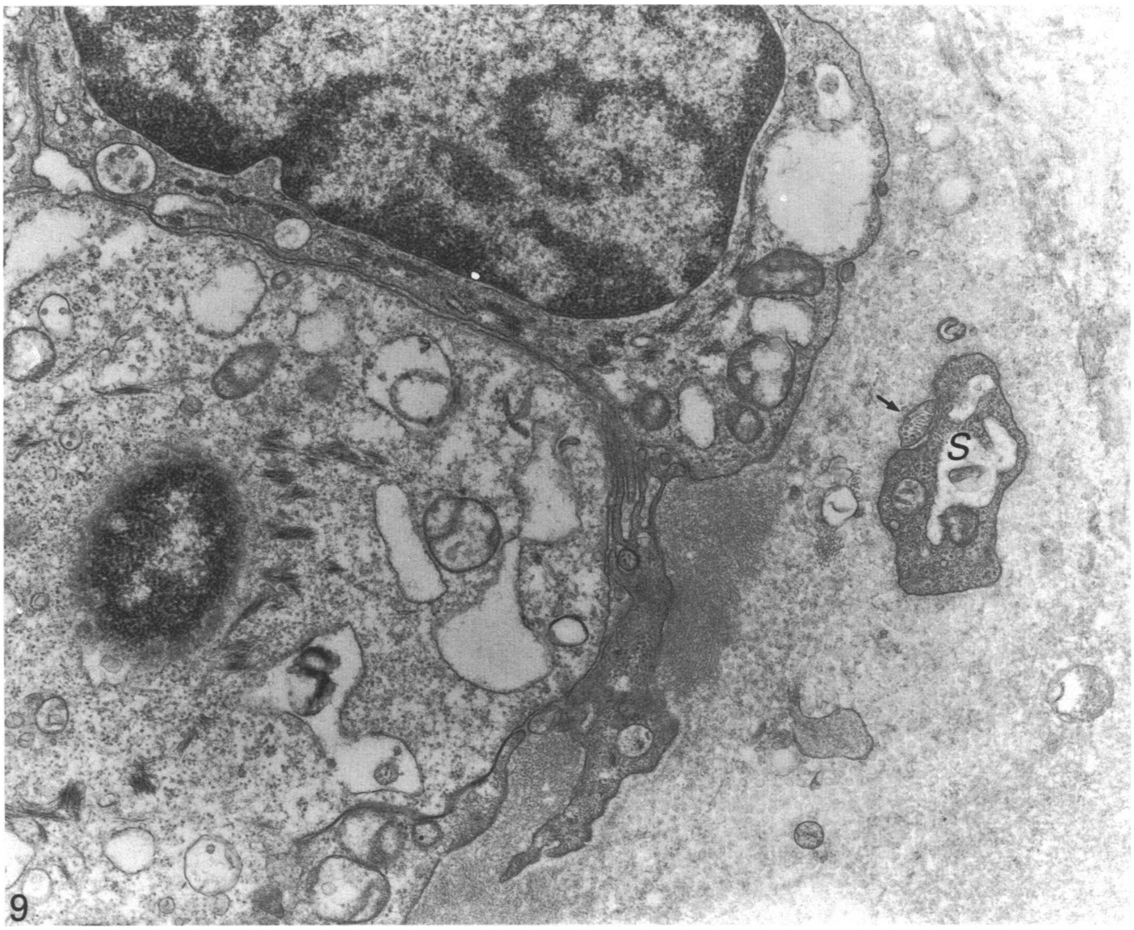

Fig. 9. Electron micrograph showing an unmyelinated axon (↑) in the immediate vicinity of an ERM. A Schwann cell process (*S*) faces the outer connective tissue. ×23500.

tions). After cryotomy and staining with NGF antibodies strong reactivity was observed in the numerous epithelial rests, neural receptor structures and in perivascular areas. NGF receptors have also been observed in pinocytotic vesicles in the membrane of Schwann cells ensheathing Ruffini-like endings in the periodontal ligament of the rat (Byers, 1990). In the present investigation, (Figs 5, 6), similar pinocytotic vesicles in the enclosing cells of Ruffini-like receptors or vesicles in the neural ending itself (Fig. 8) were observed at the level of epithelial rests of Malassez.

In conclusion, the intimate relationship between the ERM and free and Ruffini-like neural endings established in the present report probably indicates a target function towards budding nerve fibres running through the periodontal ligament.

ACKNOWLEDGEMENTS

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