Lamellar and «club-shaped» corpuscular nerve endings in human gingival mucosa. A light and electron microscopic study

P. NARDUCCI, M. ZWEYER, R. BAREGGI, M.A. SANDRUCCI, G. BALDINI, V. GRILL
Institute of Human Anatomy, University of Trieste, Italy

SUMMARY

A study on the presence of corpuscular nerve endings in human gingival mucosa was performed using both light and transmission electron microscopic (TEM) techniques. Both round and oval lamellar corpuscles were detected by light microscopy. They were located either subepithelially, close to the basement membrane, or within the papillae, deeply invaginated into the overlying epithelium. TEM techniques showed convoluted structures with unmyelinated fibre arborizations leading to an afferent fibre supported by the so called lamellar cells. The presence of blood vessels, collagenous fibrils, desmosome-like junctions, cytoplasmic organelles, as well as the similarity with some previously described mechanoreceptors, suggested the role of such corpuscular nerve endings in transmitting a nervous impulse induced by mechanical stimulation. Other simpler structures were also observed and named «club-shaped» corpuscles: they could support the more complex ones in responding to the strengths and the movements directly influencing the gingival mucosa.

KEY WORDS:

Human Gingival Mucosa - Corpuscular Nerve Endings - Histology - TEM.

RÉSUMÉ

La présence de terminaisons nerveuses corpusculaires dans la muqueuse gingivale humaine a été observée tant en microscopie optique qu'en microscopie électronique à transmission. En microscopie optique on a remarqué des corpuscules lamellaires ronds et ovalaires, qui étaient localisés tant au dessous de l'épithélium, tout près de la membrane basale, qu'au dedans des papilles, profondément insérés dans l'épithélium. En microscopie électronique on a observé des structures convolutées pourvues d'arborisations de fibres nerveuses sans myéline qui vont se réunir dans une fibre afférente supportée par des cellules dites lamellaires. La présence de vaisseaux, de fibrilles collagènes, de jonctions telles que desmoses, d'inclusions cytoplasmiques autant que la ressemblance avec quelques mécanorécepteurs décrits en littérature, suggérait un rôle de ces terminaisons nerveuses corpusculaires en envoyant un impulse nerveux induit par une stimulation mécanique. On a aussi observé des corpuscules plus simples appelés *«club-shaped»* qui pourraient supporter les plus complexes dans la réponse aux forces et aux mouvements qui influencent directement la muqueuse gingivale.

MOTS CLEFS:

Muqueuse gingivale humaine - Terminaisons nerveuses corpusculaires - Histologie - Microscopie électronique à transmission.

INTRODUCTION

Both free and corpuscular nerve endings are present in the oral mucosa for sensory perceptions. Free nerve endings respond to pain, the corpuscular ones to tactile and thermic stimulations (Holland, 1984). Many authors have already described the morphological variability of the nerve endings in the oral mucosa, both by light (Dixon, 1961, 1962; Yamamoto, 1981; Sakada, 1983) and electron microscopy (Martinez and Pekarty, 1974; Spassova, 1974; Halata and Munger, 1983; Watanabe and Yamada, 1983, 1984; Watanabe *et al.*, 1985). These studies were especially performed in animals (Martinez and Pekarty, 1974; Spassova, 1974, 1981; Saxod, 1978; Hyodo, 1979; Yamamoto, 1981; Yamamoto and Sakada, 1981, 1985; Tsukamoto, 1982; Halata and Munger, 1983; Watanabe and Yamada, 1983, 1984; Watanabe *et al.*, 1985; Tachibana *et al.*, 1987) and seldom in man (Watanabe, 1982).

Ramieri *et al.* (1990) described new fibres, corpuscles and neuroendocrine (Merkel's) cells in human oral mucosa using immunohistochemical methods.

Corpuscles in the oral mucosa have been identified as follows: Merkel's (Saxod, 1978), Meissner's corpuscles (Halata and Munger, 1983; Ramieri *et al.*, 1990), Krause's endbulbs (Spassova, 1974, 1981), Grandry's and Herbst's (Watanabe *et al.*, 1985) or merely corpuscles of «simple» and «compound» type (Tachibana *et al.*, 1987). Others have been described as lamellar nerve endings, located either in the subepithelial region of the connective tissue (Watanabe *et al.*, 1985) or in the papillar space (Martinez and Pekarty, 1974; Watanabe, 1982).

The aim of this study was to describe some morphological types of corpuscles in human gingival mucosa both by light and transmission electron microscopy (TEM).

MATERIAL AND METHODS

Twenty five specimens of healthy human gingival mucosa were obtained, from the posterior quadrants of the vestibular and lingual sides, during oral surgical procedures on agreeable patients previously anaesthesized by either mepivacaine or cryo-anaesthesia.

Samples were washed in 0.1 M phosphate buffer saline (PBS, pH 7.3), immediately fixed in 2.5% glutaraldehyde (in 0.1 M PBS, pH 7.3), cut into small pieces and maintained in the same solution for 2 h at 4°C. Postfixation was performed in 1% O_sO_4 in 0.1 M PBS (pH 7.3) for 1 h.

After dehydration in ethanol, tissue fragments were treated with propylene epoxid and finally embedded in Epon 812.

Serial semithin sections (1 μm) were cut using a Reichert Jung OmU2 ultramicrotome, then stained performing the following methods:

1) 1% toluidine blue in 0.5% Na_2CO_3 aqueous solution (Laschi and Baccarani, 1967).

2) 0.25% Azure II and 0.25% methylene blue in 0.5% Na_2CO_3 aqueous solution and then counterstained in 0.5% safranine aqueous solution (Laczkò and Lévai, 1975).

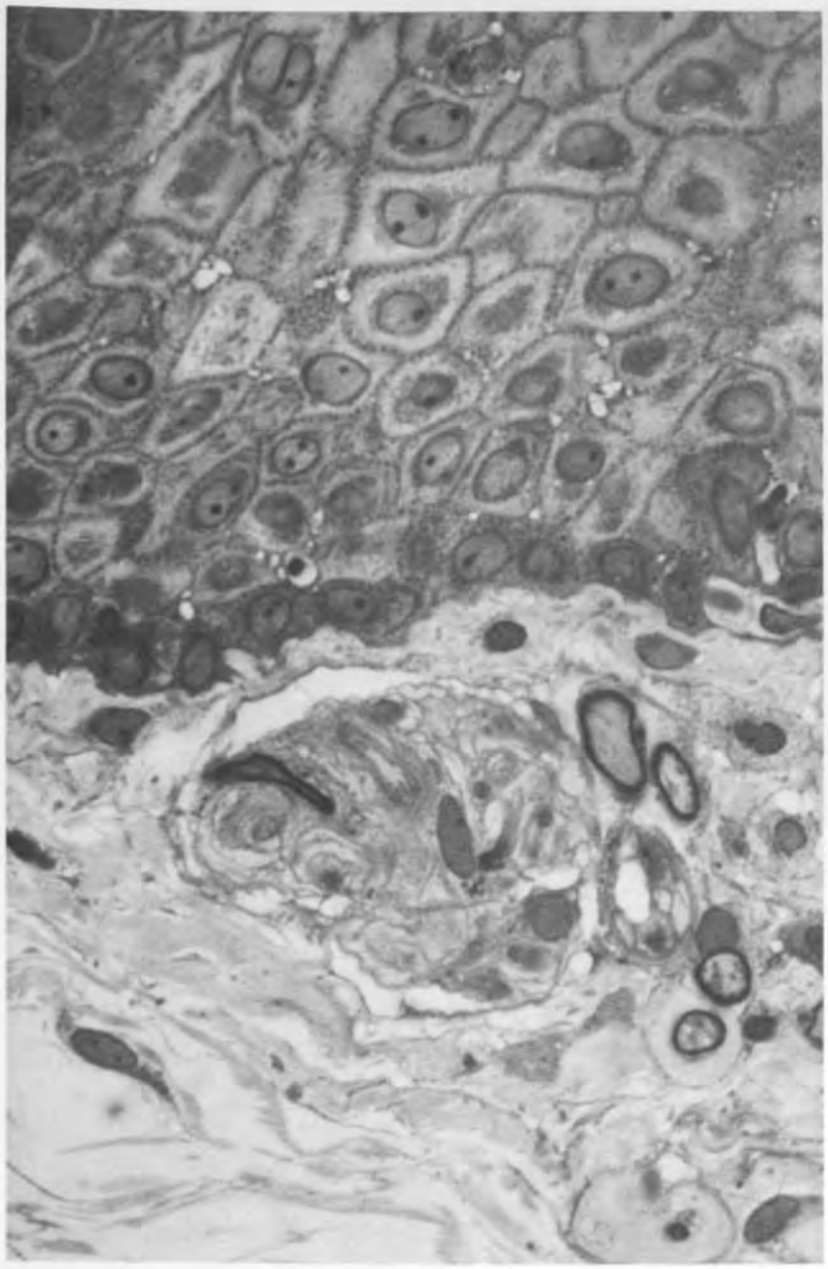
The sections were observed using a Leitz Orthoplan light microscope and photographed by a Leitz Vario-Orthomat dispositive on Agfapan 50 professional films.

Ultrathin sections (0.1 μm) were cut using a Reichert Jung Ultracut ultramicrotome, double-stained with uranyle acetate (25 min), followed by lead citrate (5 min) (Reynolds, 1963). They were examined using a Jeol Jem 100 S transmission electron microscope and photographed on Scientia Gevaert 23 D56 film.

RESULTS

Light microscopy

The light microscopic study of specimens (mainly those from the marginal gingiva) showed many lamellar nerve endings which differed in shape, size and location from the corpuscles (Krause's, Pacini's, Meissner's) most frequently found in the gingival mucosa. They were located in the connective tissue, close to the basement membrane (Fig. 1) or penetrating into the connective papillae (Fig. 2). The corpuscles consisted of nerve endings and their related accessorial structures. The examination of serial sections showed that one or more myelinated nerve fibres at first lost their myelinated sheaths, then branched in many unmyelinated nerve endings, surrounded by concentric lamellar structures. Blood vessels and nerve endings of variable size were often found close to the largest corpuscles.



Round corpuscles were rare, whereas the oval ones numerous, and their longest diameter (varying from 100 and 120 μm) was parallel to the interface line between the epithelium and the connective tissue (Fig. 3).

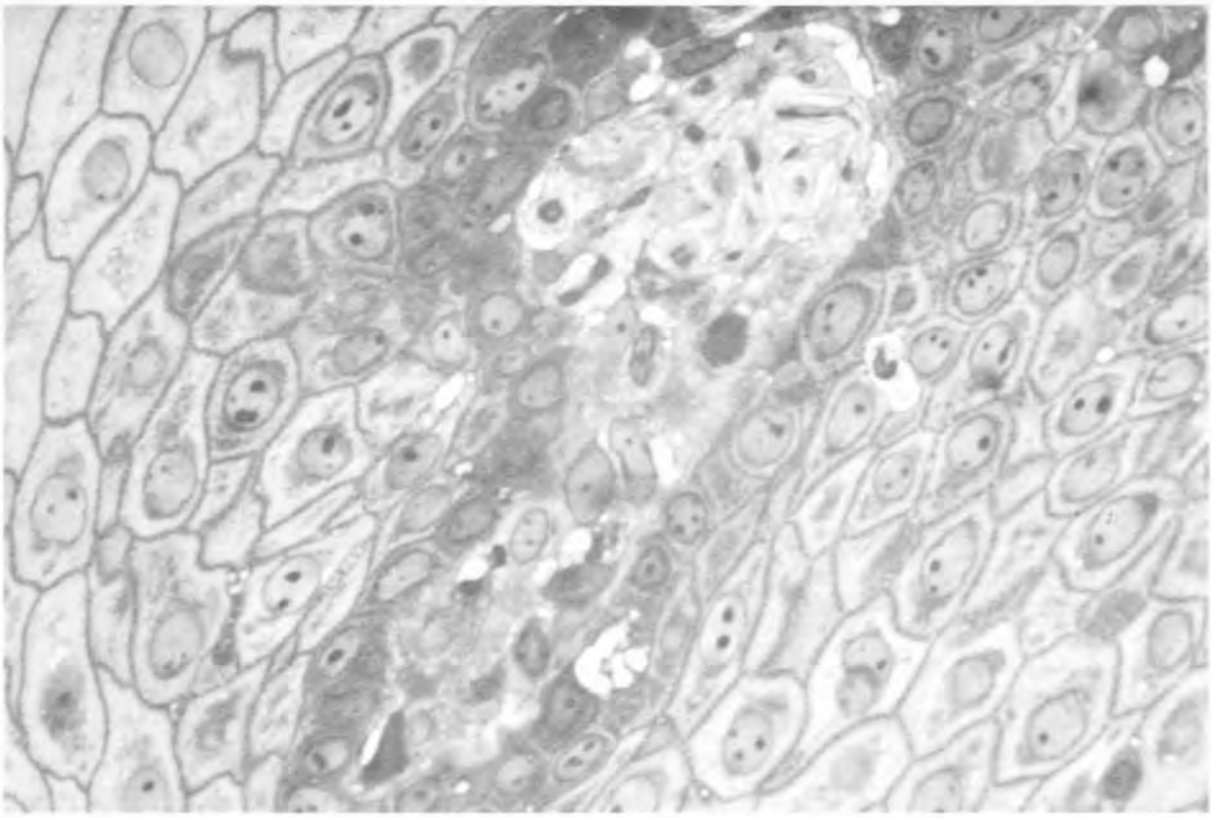
The corpuscular size depended on the number of unmyelinated nerve endings and their relative surrounding lamellar structures.

◄

Fig. 1: Lamellar sensitive corpuscle in the subepithelial area. Toluidine blue, microscopic magnification ×1000.
Fig. 1: Corpuscule sensitif lamellaire subépithélial. Bleu de toluidine (MO 1000×).

Fig. 2: Lamellar sensitive corpuscle located in an intrapapillary space. Toluidine blue, microscopic magnification ×1000.
Fig. 2: Corpuscule sensitif lamellaire intrapapillaire. Bleu de toluidine (MO 1000×).

▼



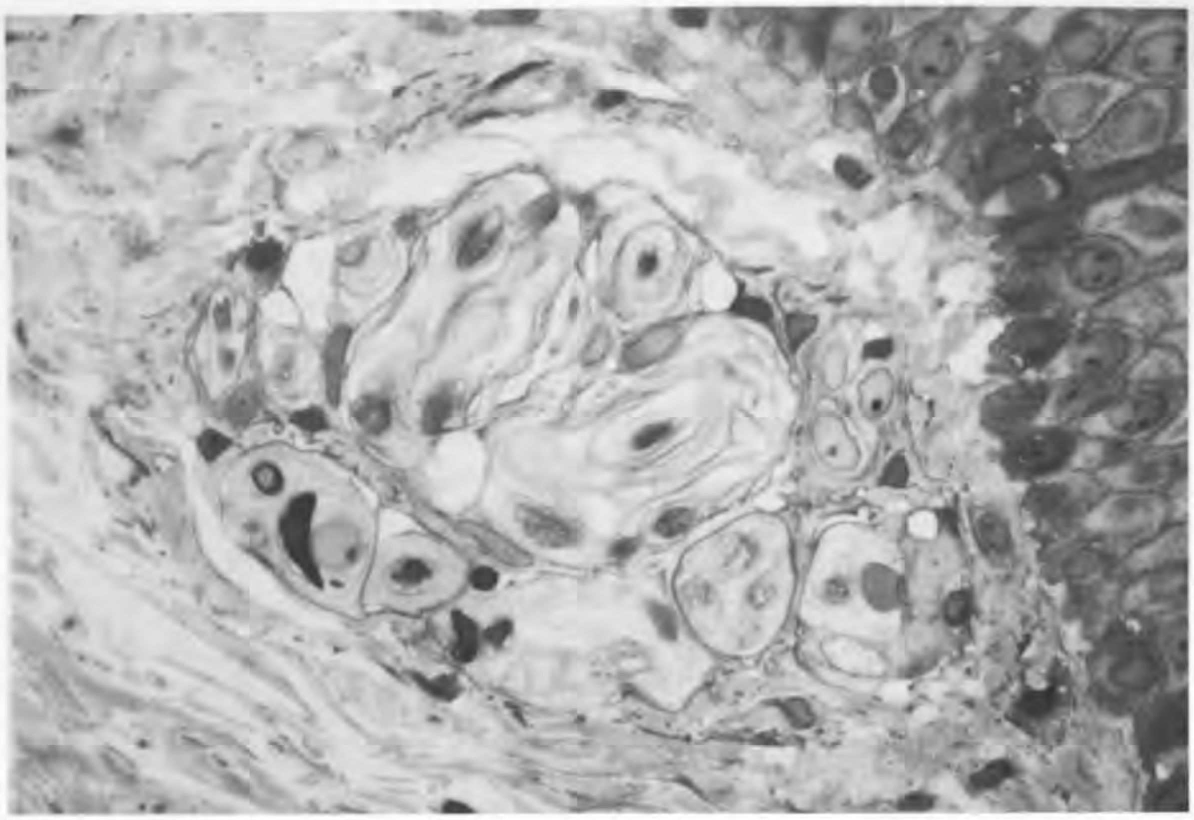


Fig. 3: Another lamellar corpuscle in the subepithelial area. Lackzò and Lévai technique, microscopic magnification ×1000.
Fig. 3: Un autre corpuscule lamellaire subépithélial. Technique de Lackzò et Lévai (MO 1000×).

TEM observations

Lamellar corpuscles

These nervous structures were formed by unmyelinated fibre arborizations surrounded by the so called lamellar cells. These expansions were very thin in proximity to the nerve endings and thicker in the outermost region (Fig. 4).

The corpuscles sometimes appeared in close contact with myelinated nerve fibres and blood capillaries. A section of a lamellar corpuscle usually contained from a minimum of 5 up to 30 unmyelinated nerve fibres surrounded by a variable number of concentric lamellar structures.

Each nerve ending showed numerous mitochondria within the axoplasm, as well as pinocytotic vesicles of variable size close to the cytoplasmic membrane (Fig. 5).

The laminar expansions of the lamellar cells presented desmosome-like junctions with the nerve fibre membrane, thus the spaces among the membrane layers were very small in this area. On the contrary the distances among the outermost laminar layers were variable: in some points, the membranes were attached, but most of them were extremely distant, sometimes reaching 100-150 nm or even more.

The extracellular matrix among the capsular cells was usually amorphous or finely granular, as well as bundles of collagenous fibrils were also present. The nuclei of the lamellar cells were regularly shaped, either oval or round, moderately electron dense and contained sparse chromatin. REG profiles were observed near the nuclei; lamellar expansions surrounding the fibre contained mitochondria, microtubules and many pinocytotic vesicles in various developmental stages, indicating an intense metabolic activity (Fig. 6). The outermost lamellae contained bundles of microfilaments deriving from desmosome-like junctions and extending into the cytoplasm.

« Club-shaped » corpuscles

Another type of nerve endings, located deeplier in the connective tissue, could be detected only by TEM (Fig. 7). They were oval and showed the largest diameter, parallel with the nerve ending axis, of about 20 μm, whereas the smallest one of about 5 μm.

The corpuscles consisted of a convoluted myelinated nerve fibre, surrounded by cells forming a capsular structure. The capsular cells appeared flattened and showed an indented nucleus in eccentric position, close to the plasmalemma. Their cytoplasm contained some REG profiles, mitochondria and clear micropinocytotic vesicles.

These structures, never previously observed to our knowledge, were named « club-shaped » corpuscles.

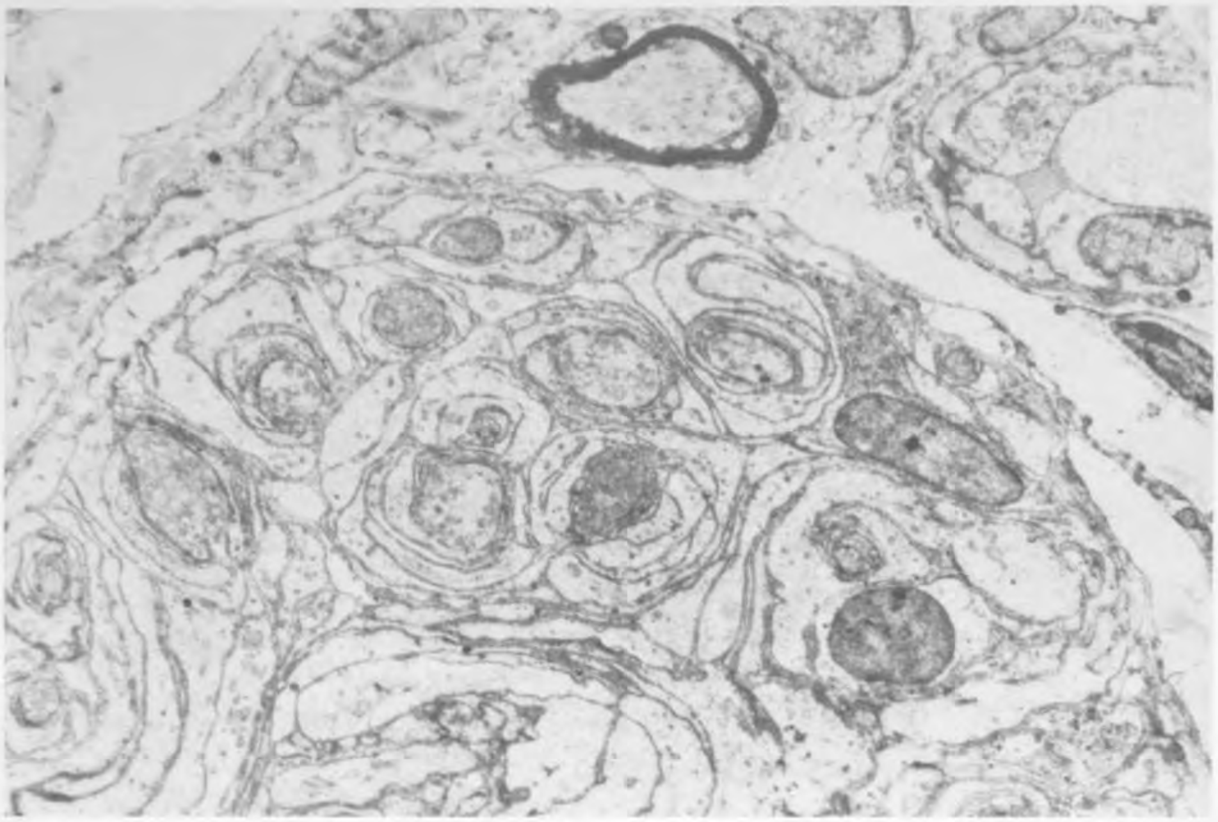


Fig. 4: TEM micrograph of a lamellar nerve ending within the connective tissue of the gingival mucosa. Microscopic magnification ×1000.
Fig. 4: Terminaison nerveuse lamellaire dans le tissu conjonctif de la muqueuse gingivale (TEM 1000×).

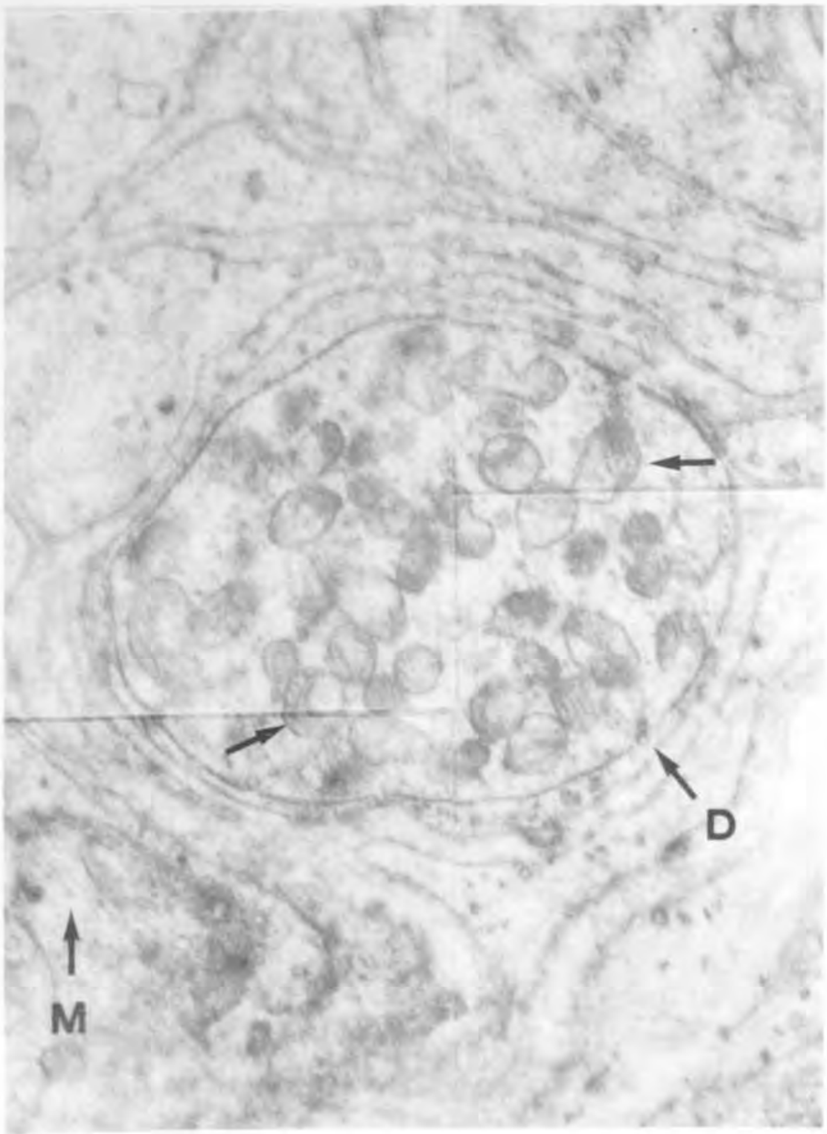


Fig. 5: TEM micrograph of an unmyelinated nerve fibre with its lamellar coils. Note mitochondria within the axoplasm (arrows), a desmosome between the fibre ans its inner coil (D), cytoplasmic microfilaments of the outer coils (M), micropinocytotic vesicles. Microscopic magnification ×15000.
Fig. 5: Fibre nerveuse sans myéline avec des cercles lamellés. On observe des mithocondres à l'intérieur de l'axoplasme (flèches), un desmose entre la fibre et les lamelles intérieures (D), des microfilaments cytoplasmiques dans les lamelles les plus extérieures (M), des vacuoles micropinocytotiques (TEM 15000×).

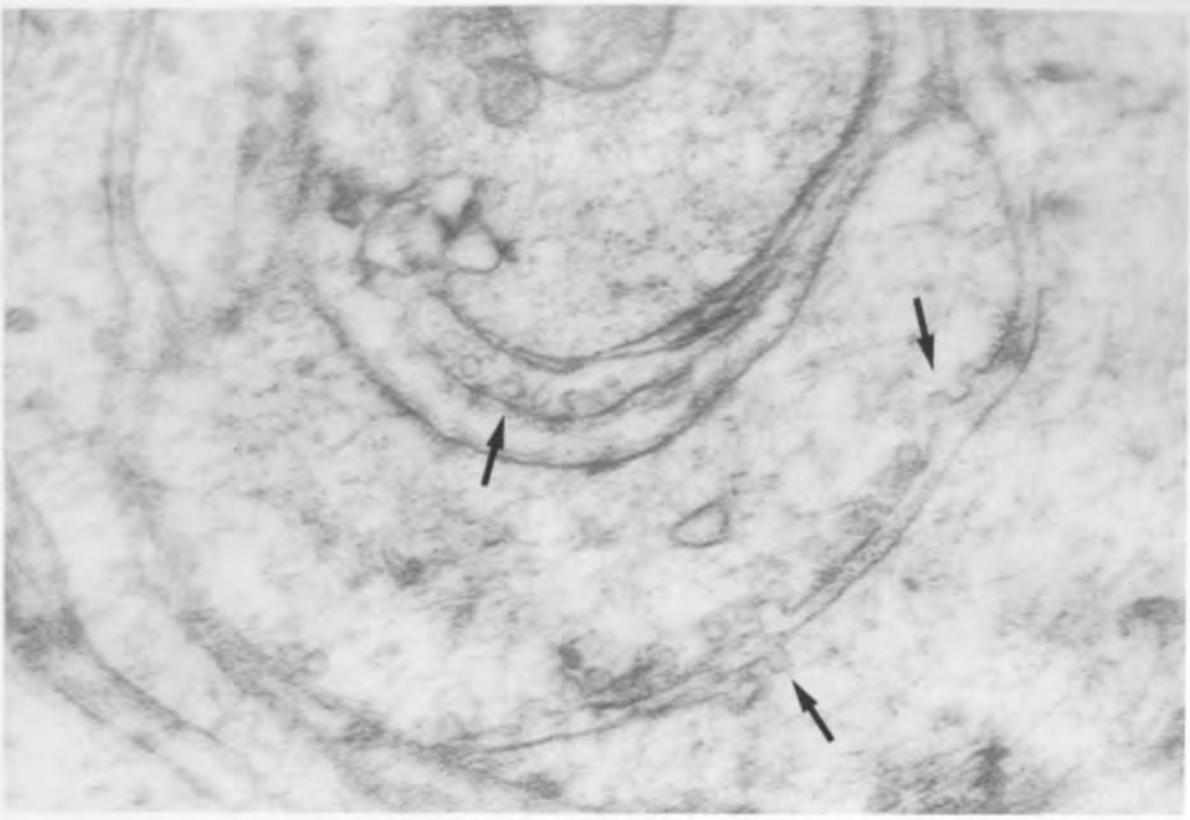


Fig. 6: TEM micrograph of micropinocytotic vesicles. Microscopic magnification ×25000.
Fig. 6: Vacuoles micropinocytotiques (TEM ×25000).

Fig. 7: TEM micrograph of a «club-shaped» corpuscle. See the terminal fibre with its myelinated bundle-like wrapping and its surrounding cells. Microscopic magnification ×4000.
Fig. 7: Corpuscule «club-shaped». On observe la fibre terminale avec un enveloppement tel qu'à myeline (TEM 4000×).

DISCUSSION

The corpuscles described in the present work, i.e. the lamellar and the «club-shaped», differed in shape and location from those previously identified in human gingival mucosa. With this regard, Ramieri *et al.* (1990) observed sensory nerve terminals mainly in the attached gingiva, whereas the present study reports their presence above all in the marginal one. Furthermore we found corpuscles in the connective tissue of both the subepithelial region and the intrapapillary space, whereas Watanabe (1982) described corpuscular nerve endings in man and in *Cebus Apella* monkey only in the intrapapillary space and never found these structures close to the basement membrane.

In rat gingiva corpuscular nerve endings, very similar to those described by us, have been reported (Martinez and Pekarty, 1974), but no collagen fibrils were present in the space among the cell layers. Watanabe and Yamada (1983) identified corpuscles like those observed by Martinez and Pekarty (1974), although in this case surrounded by collagen fibrils.

The various types of the lamellar corpuscles were morphologically similar, so that their function is probably alike too, however their anatomical location was not the same.

The role of lamellar nerve endings is not clear yet, but some of their features — i.e. the close contact to the basement membrane, the presence of collagenous fibrils within the intercellular spaces and desmosome-like junctions among the lamellar cells, as well as between these and nerve fibres — may indicate a function in the transmission of a nerve impulse induced by mechanical stimulation.

The presence of many pinocytotic vesicles in the axolemma, as well as in the cytoplasm of the corpuscular capsular cells shall be still discussed. These vesicles could merely indicate a normal metabolic activity, but it is also possible they transmit from the lamellar cells to the nerve fibre substances which are involved in nerve conduction.

In fact, the closeness of nerve endings to the lamellar cells could contribute to the reception of mechanical stimulations and their transformation into a nerve impulse. This hypothesis could be confirmed considering that some authors (Martinez and Pekarty, 1974; Spassova, 1974) had already described the above mentioned characteristics in mechanoreceptors.

In conclusion, the morphological characteristics and the presence of specific cytoplasmic organelles in the lamellar corpuscles in human gingival mucosa may suggest they could be equipped to respond appropriately to the mechanical stimulations produced by strenghts and movements directly influencing the gingiva. At the same time, the so called «club-shaped» corpuscles, not described in literature yet, could support the lamellar ones in the above mentioned functions.

ACKNOWLEDGEMENT

This work was supported by grants from the Italian Ministry for University and Scientific and Technological Research (MURST).

REFERENCES

Dixon, A.D. — Sensory nerve terminals in the oral mucosa. *Archs Oral Biol., 5:* 105-114; 1961.

Dixon, A.D. — The position, incidence and origin of sensory nerve termination in oral mucous membranes. *Archs Oral Biol., 7:* 39-48; 1962.

Halata, Z. and Munger, B.L. — The sensory innervation of primate facial skin. II. Vermilion border and mucosa of lip. *Brain Res., 286:* 81-107; 1983.

Holland, G.R. — Innervation of oral mucosa and sensory perception. In: Meyer, J., Squier, C.A., Gerson, S.J., eds. — The structure and function of oral mucosa. Oxford, Pergamon Press, pp. 196-217, 1984.

Hyodo, S. — The nerve terminals of sensory units in the oral mucosa in the cat mandible. *Shikwa Gakuho* (Japan), *79:* 1815-1836; 1979.

Laczkò, J., Lévai, G. — A simple differential staining method for semithin sections of ossifying cartilage and bone tissue embedded in epoxy resin. *Mikroskopie, 31:* 1-4; 1975.

Laschi, R., Baccarani, M. — Microscopia ottica di sezioni semifini ottenute da materiale preparato per la microscopia elettronica. *Arch. Ital. Anat. Embriol., 72:* 315-325; 1967.

Martinez, B., Pekarty, J.M. — Ultrastructure of encapsulated nerve endings in rat gingiva. *Am. J. Anat., 140:* 135-138; 1974.

Ramieri, G., Anselmetti, G.C., Baracchi, F., Panzica, G.C., Viglietti-Panzica, C., Modica, R. — The innervation of human teeth and gingival epithelium as revealed by means of an antiserum for protein gene product 9.5 (PGP). *Am. J. Anat., 189:* 146-154; 1990.

Reynolds, E.S. — The use of lead citrate at high pH as electronopaque stain in electron microscopy. *J. Cell. Biol., 17:* 208-212; 1963.

Sakada, S. — Physiology of mechanical senses of the oral structure. *Front. Oral Physiol., 4:* 1-32; 1983.

Saxod, R. — Ultrastructure of Merkel corpuscles and so-called «transitional» cells in the white leghorn chicken. *Am. J. Anat., 151:* 453-474; 1978.

Spassova, I. — Ultrastructure of the simple encapsulated nerve endings (simple end-bulb of Krause) in the tongue of the cat. *J. Anat., 118:* 1-4; 1974.

Spassova, I. — Ultrastructural relationship between the receptor nerve fiber and surrounding lamellae in Krause end bulbs. *Acta Anat., 109:* 360-368; 1981.

Tachibana, T., Ishizeki, K., Sakakura, Y. — Distinct types of encapsulated sensory corpuscles in the oral mucosa of the dog: immunohistochemical and electron microscopic studies. *Anat. Rec., 217:* 90-98; 1987.

Tsukamoto, Y. — Structure and function of encapsulated endings of single units of cats oral mucosa. *J. Tokyo Dent. Coll. Soc., 82:* 417-437; 1982.

Watanabe, I. — Fine structure of lamellated nerve endings in the gingiva of man and the *Cebus Apella* monkey. *Okajama Folia Anat., 59:* 181-193: 1982.

Watanabe, I., Yamada, E. — The fine structure of lamellated nerve endings found in the rat gingiva. *Archs Histol. Jpn., 46:* 173-182; 1983.

Watanabe, I., Yamada, E. — Unusual nerve endings found in the rat palatine mucosa. *Archs Histol. Jpn., 47:* 181-196; 1984.

Watanabe, I., Usukura, J., Yamada, E. — Electron microscope study of the Grandry and Herbst corpuscles in the palatine mucosa, gingival mucosa and beak skin of the duck. *Archs Histol. Jpn., 48:* 89-108; 1985.

Yamamoto, T. — Morphological study of the sensory nerve endings in the labial mucosa of the mouse. Morphology and distribution of the sensory nerve endings. *Shikwa Gekuho* (Japan), *81:* 933-945; 1981.

Yamamoto, T., Sakada, S. — Distribution of sensory nerve endings in the labial mucosa of the mouse. *Tohoku J. Exp. Med., 135:* 345-347; 1981.

Yamamoto, T., Sakada, S. — Distribution of sensory nerve endings in the rat labial mucosa. *Jpn. J. Oral Biol., 27:* 1245-1247; 1985.

Address: Dr. M. Zweyer, Istituto di Anatomia Umana Normale, Via Manzoni 16, I-34138 Trieste (Italy).