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THE FINE STRUCTURE OF THE DEVELOPING HUMAN PERIODONTIUM

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Summary—Electron microscopy showed that the human developing periodontium consists of synthesizing fibroblasts, collagen fibrils 300–520Å in diameter, a ground substance composed of a microfibrillar reticulum, and occasional oxytalan fibrils, 1250–3500Å in diameter.

The fibroblasts were similar to those in the dental pulp but had a more extensive development of the rough-surfaced endoplasmic reticulum. Smooth-walled vesicles in the Golgi complex and at the plasma membrane were of similar size. Cisternal vesicles, containing coiled, irregularly beaded filaments, were in close proximity to the plasma membrane. The microfibrils of 50–100Å diameter were coiled and irregularly beaded. Collagen fibrils appeared to form in association with the microfibril and an amorphous substance surrounded the beaded elements. The oxytalan fibril appeared to be composed of 30–40 microfibrils, morphologically similar to the ground substance microfibrils, lying parallel to one another and connected by lateral branches. It is suggested that the oxytalan fibril could form as a result of end to end linkage and lateral aggregations of the ground substance microfibrils and consists of a protein-polysaccharide complex. The oxytalan fibre as seen in light microscopy may correspond to bundles of oxytalan fibrils.

INTRODUCTION

IN A PREVIOUS communication the fine structure of the human developing dental pulp was reported (GRIFFIN and HARRIS, 1966; HARRIS and GRIFFIN, 1966). A prominent feature of this tissue was the presence of an extracellular reticulum consisting of coiled, irregularly beaded microfibrils or filaments in which collagen fibrils were seen. This reticulum appeared to be constituted by microfibrils which were synthesized in the dilated cisternae of dental pulp fibroblasts. It is the purpose of this paper to report the structure of a closely related tissue, namely the human developing periodontium.

MATERIAL AND METHODS

The material examined consisted of dental sacs removed from unerupted third molar teeth extracted from four patients, aged 14–16 years, under general anaesthesia. Radiographically all the teeth had reached the end of the crown formation stage of tooth development and root formation was commencing. Within 1 min of removal of the teeth the dental sacs were dissected from them as follows: The sacs were bisected

by an incision passing from the coronal to the apical part of the tooth. A mucoperiosteal elevator was then inserted between the enamel and the dental sac and the latter was readily removed from the tooth. The material was then covered with Palade's osmium tetroxide fixative at 0°C. Whilst under cover of the fixative the sacs were divided into three portions: one from the coronal part of the tooth, one from the apical part and one from the mid-crown portion. The portions from the apical and mid-crown parts were cut into 0·5 × 1·0 mm pieces which were then placed in tubes of Palade's osmium tetroxide fixative at 0°C for 4 hr. After fixation, the pieces were dehydrated in increasing concentrations of acetone (25, 50 and 75% for 15 min each) and, after rinsing in three changes of acetone, were embedded in Araldite. Polymerisation was effected at 60°C for 24 hr. Thin sections were cut on an L.K.B. ultramicrotome and stained on the grid with uranyl acetate for 1 hr. Electron micrographs and observations were made on Hitachi H.S.7 and Hitachi HU-IIB electron microscopes.

RESULTS

(a) *Fibroblasts*

Active periodontal fibroblasts were essentially similar to dental pulp synthesizing fibroblasts (GRIFFIN and HARRIS, 1966) except that there appeared to be more extensive development of rough-surfaced endoplasmic reticulum (Figs. 1 and 2). The numerous dilated cisternae associated with the rough-surfaced endoplasmic reticulum contained irregularly delineated electron-dense material which at intervals exhibited more electron-dense oval masses (beaded elements). These structures have been termed by us coiled, irregularly beaded microfibrils (Figs. 3 and 4).

The fibrillar component consisted of slender, not clearly delineated, elongated and coiled material of variable diameter (50–100Å). The beaded components were measured and the mean diameter of thirty of them was 200Å (standard deviation 28·9 and a coefficient of variation 14·5%).

The Golgi complex consisted of distended, smooth-walled sacs, aggregations of smooth-surfaced, double layered membranes arranged parallel to each other, and numerous small, smooth-walled vesicles (Fig. 5). These elements contained an amorphous, moderately electron-dense material. The diameters of 111 of the small, smooth-walled vesicles were measured and they were found to have a mean diameter of 564·5Å (standard deviation 103·6 and a coefficient of variation 17·9%). A graph of their distribution on probability paper showed a normal distribution.

Vesicles similar in appearance to the small, smooth-walled vesicles of the Golgi complex were seen aggregated at the plasma membrane (Fig. 3). The diameters of 33 of these vesicles were measured and they were found to have a mean diameter of 568·8Å (standard deviation 128·6 and a coefficient of variation 22·6%). A graph of their distribution on probability paper showed a normal distribution. The small, smooth-walled vesicles aggregated at the plasma membrane and the small, smooth-walled vesicles of the Golgi complex were found to be not significantly different in size at a 5% level. These findings suggest that the small, smooth-walled vesicles seen

at the plasma membrane are derived from the Golgi complex. The vesicles have been termed secretory vesicles (GOLDBERG and GREEN, 1964; GRIFFIN and HARRIS, 1966).

A vesicle which has been termed the cisternal vesicle (ROSS and BENDITT, 1965; GRIFFIN and HARRIS, 1966) was also seen (Fig. 3). These vesicles when intracellular usually had attached ribosomes and contained coiled, irregularly beaded microfibrils similar in appearance to those within dilated cisternae. They were seen in close proximity to the plasma membrane (Fig. 3). There was some evidence which suggested that these vesicles pass through the plasma membrane losing their ribosomal components and then disrupting extracellularly (Figs. 3 and 6). However, we cannot be certain on this point.

(b) *Extracellular substance*

Extracellular, coiled, irregularly beaded microfibrils were seen (Figs. 4, 6 and 7). They were of indefinite length and had diameters between 50–100Å. The diameters of 32 of the beaded elements were measured and they were found to have a mean diameter of 198·4Å (standard deviation 22·9 and a coefficient of variation 11·5%). The diameters of the beads on the extracellular and intracisternal fibrils were not significantly different at a 5% level and it is therefore considered that the beaded microfibrils are synthesized in the dilated cisternae of the rough-surfaced endoplasmic reticulum and that they reach the extracellular substance by means of cisternal vesicles. An amorphous substance was usually seen to be associated with the beaded elements (Figs. 7 and 8).

These microfibrils were connected to adjacent microfibrils by lateral branches (Fig. 7) and formed a reticulum.

Short lengths of striated material about 200Å in diameter, presumably young collagen, appeared to be associated with the microfibrillar reticulum (Fig. 7). Collagen fibrils with diameters 300–350Å when sectioned transversely and longitudinally showed branches of the microfibrillar reticulum running parallel to and at right angles to their long axes (Figs. 9 and 10). Amorphous material of the microfibrillar reticulum appeared to be associated with the collagen fibrils (Figs. 9 and 10) and is probably the cause of the electron density of the young fibrils. These fibrils had a macroperiod of approximately 430Å and a microperiod consisting of 9–10 bands. Collagen fibrils with diameters of 520Å were also seen (Fig. 11). These fibrils had a macroperiod of approximately 500Å and a microperiod of 10–11 bands. The difference between the periodicity of the 300–350Å and the 520Å fibrils can be seen in Fig. 12. The larger fibrils were markedly less electron-dense than the smaller fibrils and this appeared to be due to a reduction of the associated microfibrillar reticulum (Figs. 9 and 11).

Oxytalan fibrils, which to a certain extent correspond with the oxytalan fibre as described by CARMICHAEL and FULLMER (1966), were seen. They were of indefinite length and their diameters varied between 1250–3500Å (Figs. 13–16). They consisted of irregularly beaded microfibrils which lay roughly parallel to each other and which were approximately 125Å apart. The diameters of these microfibrils varied between 75–100Å and the diameters of the beaded elements between 150–175Å. The microfibrils appeared to be connected by lateral branches and they had no obvious perio-

dicity (Figs. 13–15). In transverse section (Fig. 14) the fibrils were seen to be composed of 30–40 microfibrils of different diameters, as noted above, and it is probable that the larger diameters correspond to the beaded elements seen in the longitudinal sections. Amorphous material concentrated around the beaded elements was also seen (Fig. 14).

These oxytalan fibrils were seen near the plasma membrane (Fig. 16), isolated in the ground substance (Fig. 7), in pairs (Fig. 15) and between collagen fibres (Fig. 16). It is possible that the oxytalan fibre seen with light microscopy corresponds to bundles of the oxytalan fibrils.

DISCUSSION

(a) *Fibroblasts*

The most conspicuous feature of the synthesizing periodontal fibroblast was the presence of numerous dilated cisternae containing coiled microfibrils, cisternal vesicles and secretory vesicles. The dental pulp fibroblast at approximately the same stage of development contained similar elements (GRIFFIN and HARRIS, 1966). These microfibrils are apparently synthesized in the dilated sacs of the rough-surfaced endoplasmic reticulum. Their presence in cisternal vesicles and the location of the latter at cell membranes and extracellularly suggests that microfibrils reach the exterior of the cell by vesicular transport (Figs. 3, 4, and 6). Secretory vesicles on the other hand usually contained a moderately electron-dense amorphous substance similar to that present in the distended smooth-walled sacs of the Golgi complex (Figs. 3 and 5). These vesicles also appeared to release their contents at plasma membranes or extracellularly. ROSS and BENDITT (1965) found that vesicles which they thought might be of cisternal origin and cytoplasmic vesicles which they thought were derived from the Golgi complex contained labelled proline. Their evidence suggested that several proteins could be synthesized in the endoplasmic reticulum at different rates; some of these might then pass to the Golgi complex, and some might be dispersed elsewhere inside or outside the cell. Our observations suggest that coiled microfibrils are synthesized intracellularly and that they leave the cell principally by cisternal vesicles, whereas secretory vesicles presumably derived from the Golgi complex contain an amorphous substance, some of which might be soluble collagen.

Plasma membranes of active periodontal fibroblasts were usually intact (Figs. 1 and 2). ROSS and BENDITT (1961, 1965) and GOLDBERG and GREEN (1964) also reported that the plasma membranes of fibroblasts are usually intact and suggested that interruptions in these membranes, as observed by other authors, were the result of manipulation. There was no evidence that intracytoplasmic filaments passed out of the cell through the plasma membrane.

(b) *Extracellular Substance*

1. *Observations on ground substance microfibrils.* Ground substance microfibrils appeared to form a reticulum with which collagen fibrils and oxytalan fibrils were associated. The microfibril was found to have a preferred orientation at right angles and parallel to collagen fibrils. The amorphous component of the microfibrils

appeared to associate with the collagen fibrils. They were morphologically similar to microfibrils seen within the dilated sacs of the rough-surfaced endoplasmic reticulum and within cisternal vesicles. Microfibrils of a similar nature have been described by other authors. CHAPMAN (1961), in tissue stained with 1% phosphotungstic acid, reported the presence of densely stained clumps of partly amorphous and partly filamentous material in which short lengths of striated collagen fibrils could be recognized. He also noted the presence of irregularly beaded microfibrils which were associated with fibrillogenesis. LOW (1962) described microfibrils which were about 100Å in diameter and which were aggregated around basement membranes and elastic fibres. AYER (1964) suggested that these microfibrils might aggregate together to form elastic fibres.

2. *Observations on collagen fibrils*. The diameters of collagen fibrils varied between 200 and 600Å and they exhibited a macroperiodicity of 420–500Å and a microperiodicity of between 9 and 11 bands. The larger fibril has more bands per macroperiod. This arrangement suggests an alteration in the stagger of the collagen macromolecules as the fibril matures. The diameters of the collagen fibrils were larger (300–500Å) than that (300Å) stated by ASTBURY (1958) and also larger than those in the periodontium of the white rat (THILANDER, 1961).

The extracellular microfibrillar reticulum was seen to be associated with young collagen fibrils and with collagen fibrils aggregating to form a collagen fibre (Figs. 7, 9 and 10).

There appeared to be more microfibrillar material associated with smaller collagen fibrils than with larger collagen fibrils. This gave a more electron-dense appearance to the smaller fibrils. CHAPMAN (1961), CASTOR and MUIRDEN (1964) and GRIFFIN and HARRIS (1966) noted fibrillar material and an amorphous substance in association with young collagen fibrils and FITTON JACKSON (1964) stated that there was a relative reduction in the perifibrillar material associated with collagen fibrils as the fibrils aged.

3. *The circumstantial evidence suggesting that the microfibrils might be protein-polysaccharides*. MELCHER (1965) noted, in gingival tissue, the presence of small collagen fibrils embedded in a matrix which contained a network of electron-dense material and thought that this network might comprise finely divided collagen, or alternatively, might coincide with the protein fraction of the glycoprotein described by SNELLMAN (1963). HARRIS and GRIFFIN (1966) observed microfibrils in the developing dental pulp which are morphologically similar to periodontal microfibrils and which are associated with fibrillogenesis.

MATHEWS (1965), using electrophoresis, proposed a model for protein-polysaccharide complexes which associated with collagen fibrils. This model consisted of a core protein to which were attached about 60 side chains of chondroitin sulphate. Presumably the polysaccharide moiety was either associated or linked with collagen fibrils. SCHWARZ (1957) proposed that the argyrophilia associated with collagen was due to the presence of a polysaccharide which, as fibrils aged, decreased in amount and CASTOR and MUIRDEN (1964) were able to recover a carbohydrate substance from the

interfibrillar material. GRIFFIN and HARRIS (1966) found that in developing human dental pulp isolated collagen fibrils and collagen fibrils aggregating to form a collagen fibre were markedly more electron-dense than fibrils constituting a mature collagen fibre. Moreover, fibrils aggregating to form bundles of collagen fibrils were more electron-dense than fibrils in the centre of the bundle. These findings suggest that a protein-polysaccharide complex may be associated with collagen fibrils from their nucleation to their maturation and that part of this complex decreases as the fibrils mature. Our observations suggest that it is the amorphous component of the microfibril which is responsible for the electron density of maturing collagen fibrils (Fig. 9) and that in the more mature collagen fibril this amorphous component is reduced (Fig. 11). In this respect WOOD (1964) has suggested that there is a discrete fraction of soluble collagen which goes to form fibril nuclei and that this fraction preferentially associates with a protein-polysaccharide complex. He thought that this protein-polysaccharide complex might be covalently linked to the collagen molecules (or to a fraction of them) or it might be associated with them by secondary forces. It is possible that the microfibrils associated with collagen in the developing periodontium could correspond to a protein-polysaccharide complex. If this suggestion were true, the amorphous component could correspond to the polysaccharide moiety of the microfibril and the microfibril to the protein moiety while the beaded element could correspond to a linkage protein. Such a structure has been postulated by FITTON JACKSON (1964).

4. *Fibrillogenesis.* We would like to suggest that the probable sequence of events as regards fibrillogenesis in the developing human periodontium could be initial synthesization of microfibrils and soluble collagen in the ergastoplasmic cisternae of active fibroblasts. Microfibrils could preferentially leave the cell by cisternal vesicles and soluble collagen could preferentially leave the cell by secretory vesicles. Extracellularly the amorphous component of microfibrils could enter into an association with the collagen molecules or fraction of them and collagen fibrils could then form in association with ground substance microfibrils. The microfibrils appear to constitute an anastomosing system with a preferred orientation to collagen fibrils and might be capable of organising them into collagen fibres (HARRIS and GRIFFIN, 1966).

5. *Oxytalan fibrils.* Oxytalan fibrils seen by us differ from those described by CARMICHAEL and FULLMER (1966) in the following respects:

(i) The diameter of the fibrillar component of the oxytalan fibril as described by them was between 150–160Å, whereas the diameter observed by us indicates that it lies between 75–150Å.

(ii) The amorphous component we found was associated with the beaded element of the oxytalan microfibril, whereas they did not identify an amorphous component between the microfibrils but noted the presence of ground substance in much the same quantity as in the bundles of collagen.

(iii) Beaded elements were not observed by them.

These differences might be attributable to the fact that the material examined by CARMICHAEL and FULLMER had been decalcified.

The amorphous component presumably corresponds to the polysaccharide moiety of the oxytalan fibril, whilst the fibrillar component corresponds to the protein moiety, as inferred from results obtained by histochemical investigation (FULLMER and LILLIE, 1958).

The oxytalan microfibril (Figs. 13 and 15) is morphologically similar to the ground substance microfibril (Figs. 7 and 8) and could possibly be formed as a result of end to end linkage and lateral aggregations of these elements.

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Résumé—La microscopie électronique permet de montrer que le ligament alvéolo-dentaire, en voie de développement, présente des fibroblastes actifs, des fibrilles collagènes, de 300–520 Å de diamètre, une substance fondamentale formée par un réticulum microfibrillaire et quelques fibrilles oxytalanes, de 1250–3500 Å de diamètre.

Les fibroblastes sont identiques à ceux de la pulpe dentaire mais présentent un développement plus poussé de l'ergastoplasme à surfaces rugueuses. Des vésicules lisses de l'appareil de Golgi sont de taille identique à des vésicules situées près de la membrane cytoplasmique. Des vésicules ergastoplasmiques, contenant des filaments enroulés et irrégulièrement moniliformes, sont proches de la membrane cytoplasmique. Les microfibrilles ont un diamètre de 50–100 Å. Les fibrilles collagènes semblent se former en association avec les microfibrilles et une substance amorphe paraît circonscrire les éléments moniliformes.

La fibre oxytalane est composée de 30–40 microfibrilles, semblables au point de vue morphologique aux microfibrilles de la substance fondamentale, disposées parallèlement les unes aux autres et reliées par des branches latérales. Il semble que la fibrille oxytalane se développe par agrégation latérale et par mise bout à bout des microfibrilles de la substance fondamentale. Elle paraît constituée par un complexe protéino-polysaccharidique. La fibre oxytalane, visible en microscopie optique, pourrait correspondre aux faisceaux de fibrilles oxytalanes.

Zusammenfassung—Elektronenmikroskopisch wurde nachgewiesen, daß das in der Entwicklung befindliche menschliche Parodontium aus synthetisierenden Fibroblasten, Kollagenfibrillen im Durchmesser von 300–520 Å, sowie aus einer Grundsubstanz besteht, die sich aus einem mikrofibrillären Retikulum und gelegentlich Oxytalanfasern mit 1250–3500 Å durchmesser zusammensetzt.

Die Fibroblasten waren denen der Zahnpulpa sehr ähnlich, zeigten jedoch eine ausgeprägt rauhe Oberfläche des endoplasmischen Retikulums. Die glattwandigen Bläschen im Golgi-Apparat und an der Plasma-Membran waren von ähnlicher Größe. Cisternale Bläschen mit gewundenen, irregulär aufgereihten Achsenfäden lagen in unmittelbarer Nähe der Plasma-Membran. Die Mikrofibrillen mit 50–100 Å Durchmesser waren aufgespult und unregelmäßig aufgereiht. Die Kollagenfibrillen schienen sich in Verbindung mit den Mikrofibrillen zu bilden. Die aufgereihten Elemente waren von amorphen Substanzen umgeben. Es wurde festgestellt, daß die Oxytalanfibrillen aus 30–40 Mikrofibrillen zusammengesetzt sind, ähnlich den Mikrofibrillen der Grundsubstanz, die zueinander parallel liegen und durch laterale Abzweigungen miteinander verbunden sind. Es kann angenommen werden, daß die Oxytalanfibrillen aufgrund ihrer Endverkettung und lateralen Verbindungen in der Lage sind, in der Grundsubstanz Mikrofibrillen zu bilden, und daß sie aus einem Proteinpolysaccharid-Komplex bestehen. Die Oxytalanfibrillen können also lichtmikroskopisch besehen den gebündelten Oxytalanfibrillen entsprechen.

REFERENCES

ASTBURY, W. T. 1958. Fundamentals of collagen. *Dent. Practit.* **8,** 261–266.
AYER, J. P. 1964. Elastic tissue. *Int. Rev. connect. Tissue Res.* **2,** 33–100.
CARMICHAEL, G. G. and FULLMER, H. M. 1966. The fine structure of the oxytalan fiber. *J. Cell Biol.* **28,** 33–36.
CASTOR, C. W. and MUIRDEN, D. 1964. Collagen formation in monolayer cultures of human fibroblasts: the effect of hydrocortisone. *Lab. Invest.* **13,** 560–574.
CHAPMAN, J. A. 1961. Morphological and chemical studies of collagen formation. I. The fine structure of guinea pig granulomata. *J. biophys. biochem. Cytol.* **9,** 639–651.
FITTON JACKSON, S. 1964. *The Cell.* 6. *Connective Tissue Cells.* (Edited by BRACHET, J. and MIRSKY, A. E.), pp. 387–509. Academic Press, New York.
FULLMER, H. M. and LILLIE, R. D. 1958. The oxytalan fiber. A previously undescribed connective tissue fiber. *J. Histochem. Cytochem.* **6,** 425–430.
GOLDBERG, B. and GREEN, H. 1964. An analysis of collagen secretion by established mouse fibroblast lines. *J. Cell Biol.* **22,** 227–258.
GRIFFIN, C. J. and HARRIS, R. 1966. Ultrastructure of collagen fibrils and fibroblasts of the developing human dental pulp. *Archs oral Biol.* **11,** 659–666.
HARRIS, R. and GRIFFIN, C. J. 1967. Histogenesis of fibroblasts in human dental pulp. *Archs oral Biol.* **12,** 459–468.
LOW, F. N. 1962. Microfibrils: fine filamentous components of the tissue space. *Anat. Rec.* **142,** 131–137.
MATHEWS, M. B. 1965. The interaction of collagen and acid mucopolysaccharides. *Biochem. J.* **96,** 710–716.
MELCHER, A. H. 1965. The nature of the 'basement membrane' in human gingiva. *Archs oral Biol.* **10,** 785–794.
ROSS, R. and BENDITT, E. P. 1961. Wound healing and collagen. I. Sequential changes in components of guinea pig skin wounds observed in the electron microscope. *J. biophys. biochem. Cytol.* **11,** 677–700.
ROSS, R. and BENDITT, E. P. 1965. Wound healing and collagen formation. V. Quantitative electron microscope radioautograph observations of proline-H^3 utilization by fibroblasts. *J. Cell Biol.* **27,** 86–106.
SCHWARZ, W. 1957. Morphology and differentiation of the connective tissue fibres. In: *Connective Tissue* (Edited by TUNBRIDGE, R. E.), pp. 144–156. Blackwell, Oxford.
SNELLMAN, O. 1963. A glycoprotein from reticulum tissue. *Acta chem. scand.* **17,** 1049–1056.
THILANDER, H. 1961. Periodontal disease in the white rat. *Trans R. Sch. Dent., Stockholm & Umea.* Series No. 6, 66–71.
WOOD, G. C. 1964. The precipitation of collagen fibers from solution. *Int. Rev. connect. Tissue Res.* **2,** 1–28.

PLATE 1

FIG. 1. Synthesizing periodontal fibroblast and extracellular substance. N, nucleus; n, nucleolus; G, Golgi complex; er, dilated cisternae; P, cytoplasmic process of fibroblast; mf, ground substance microfibrils; c, collagen fibrils; Ox, oxytalan fibril. Osmium fixation, uranyl acetate. ×4375.

FIG. 2. Synthesizing periodontal fibroblast and extracellular substance. N, nucleus; n, nucleolus; G, Golgi complex; er, dilated cisternae; P, cell process; c, collagen fibrils; Ox, oxytalan fibrils; mf, ground substance microfibrils. Osmium fixation, uranyl acetate. ×4375.

FIG. 3. Cytoplasm of synthesizing periodontal fibroblast. Rough-surfaced endoplasmic reticulum (er) around dilated cisternae which contain coiled microfibrils; if, intracytoplasmic filaments; m, swollen mitochondria; intracellular secretory vesicles (arrows); vc, cisternal vesicles; pm, plasma membrane. Note amorphous material concentrated near the plasma membrane. Osmium fixation, uranyl acetate. ×33,075.

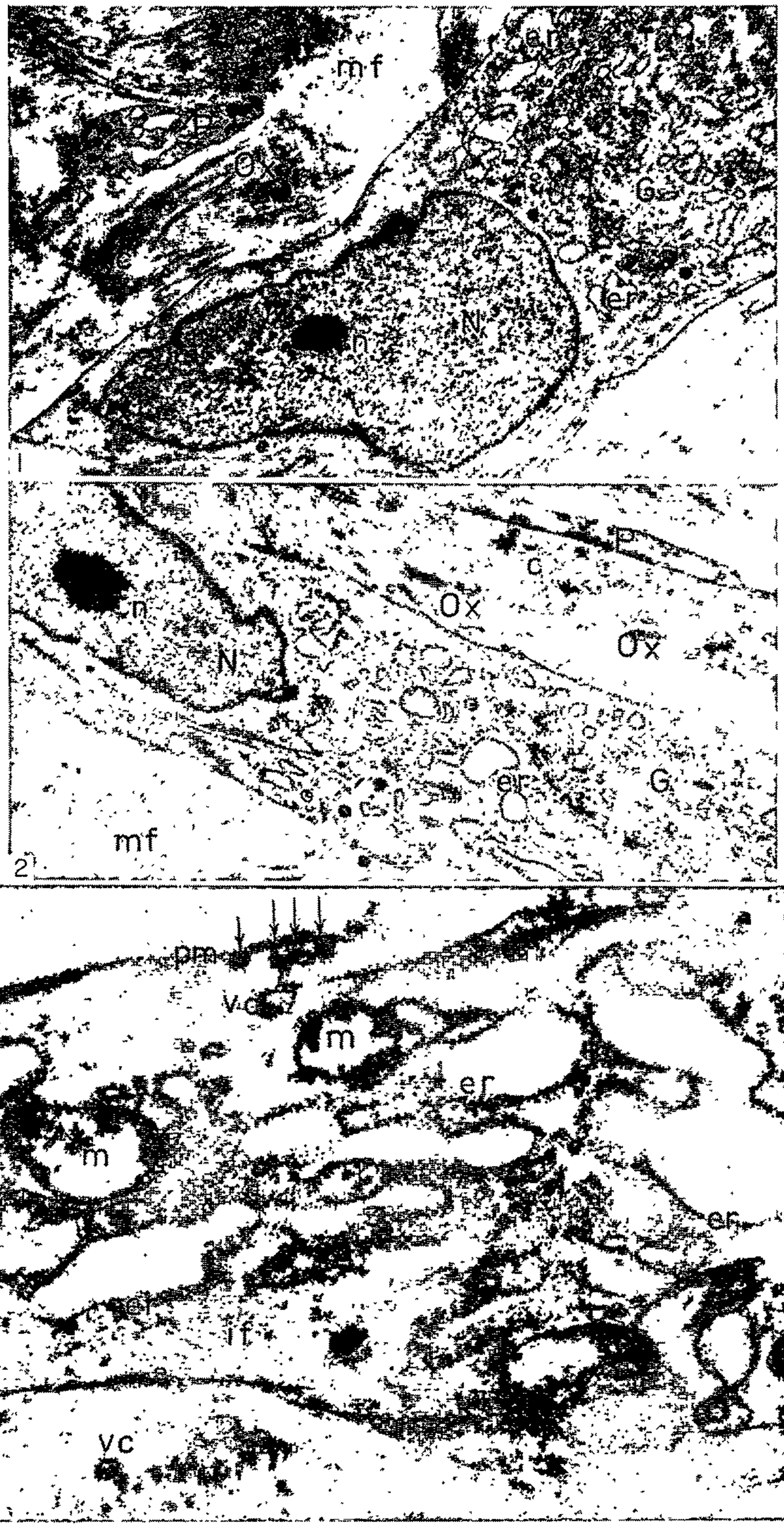


PLATE 1

A.O.B. f.p. 978

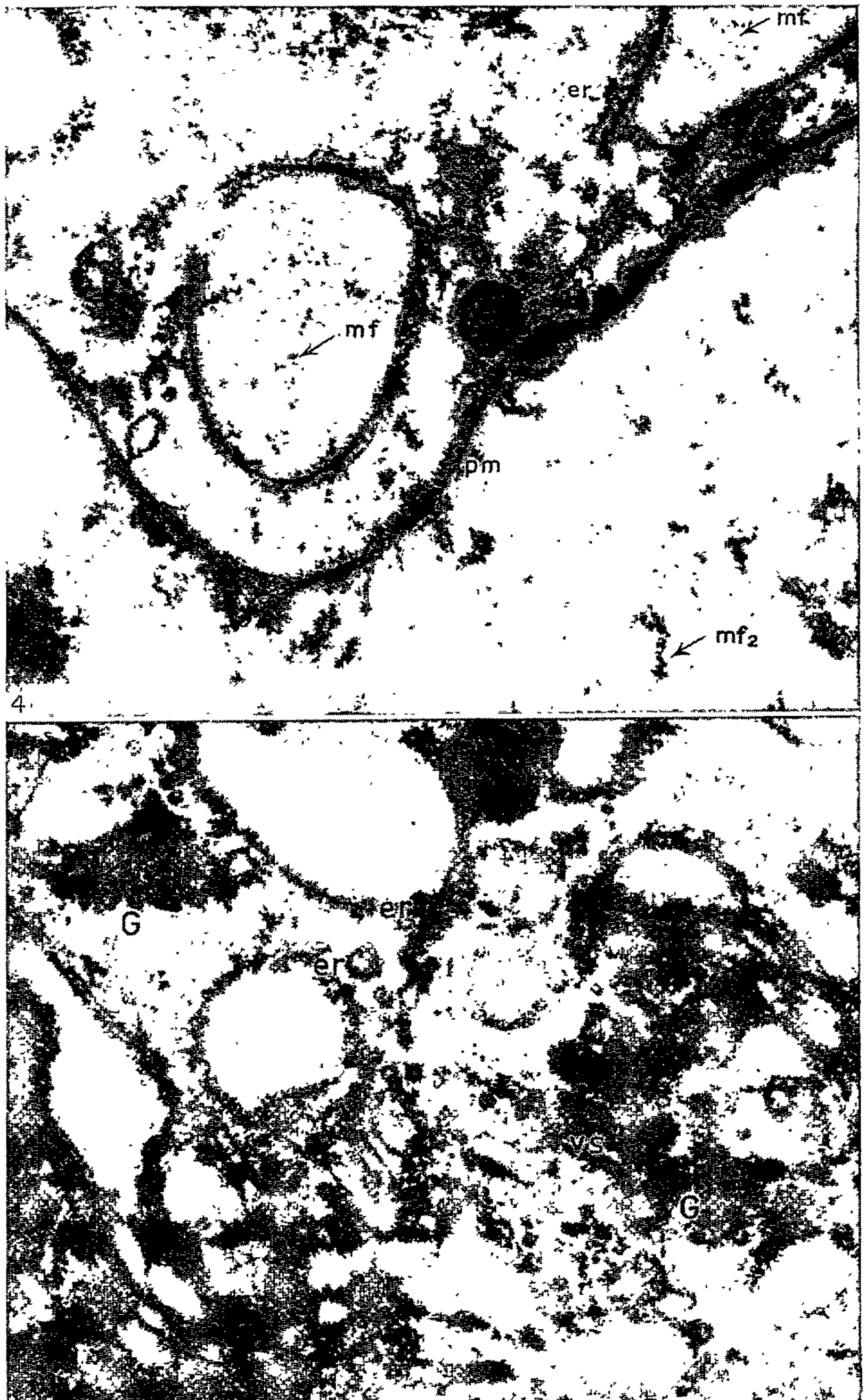


PLATE 2

PLATE 2

FIG. 4. Cytoplasm of synthesizing fibroblast and extracellular substance. er, rough-surfaced endoplasmic reticulum; mf, intracisternal microfibrils; mf_2, extracellular microfibrils; pm, plasma membrane. Osmium fixation, uranyl acetate. ×49,000.

FIG. 5. Golgi complex of synthesizing periodontal fibroblast. G, Golgi complex; er, rough-surfaced endoplasmic reticulum which in some areas is devoid of ribosomes; vs, secretory vesicles. Osmium fixation, uranyl acetate. ×39,900.

PLATE 3

FIG. 6. Portion of cytoplasm of synthesizing periodontal fibroblast and extracellular substance. er, rough-surfaced endoplasmic reticulum; pm, plasma membrane; mf, microfibrils; vc, disrupted cisternal vesicle; c, collagen fibrils in longitudinal and transverse section. Osmium fixation, uranyl acetate. ×29,400.

FIG. 7. Extracellular substance of the developing periodontium. Ox, bundles of oxytalan fibrils; c, isolated collagen fibril; mf, microfibril; amorphous material associated with beaded elements of microfibrils (arrows). Osmium fixation, uranyl acetate. ×63,000.

FIG. 8. Periodontal microfibril. pm, plasma membrane; a, fibrillar component; b, beaded elements; amorphous component (arrow). Osmium fixation, uranyl acetate. ×145,600.

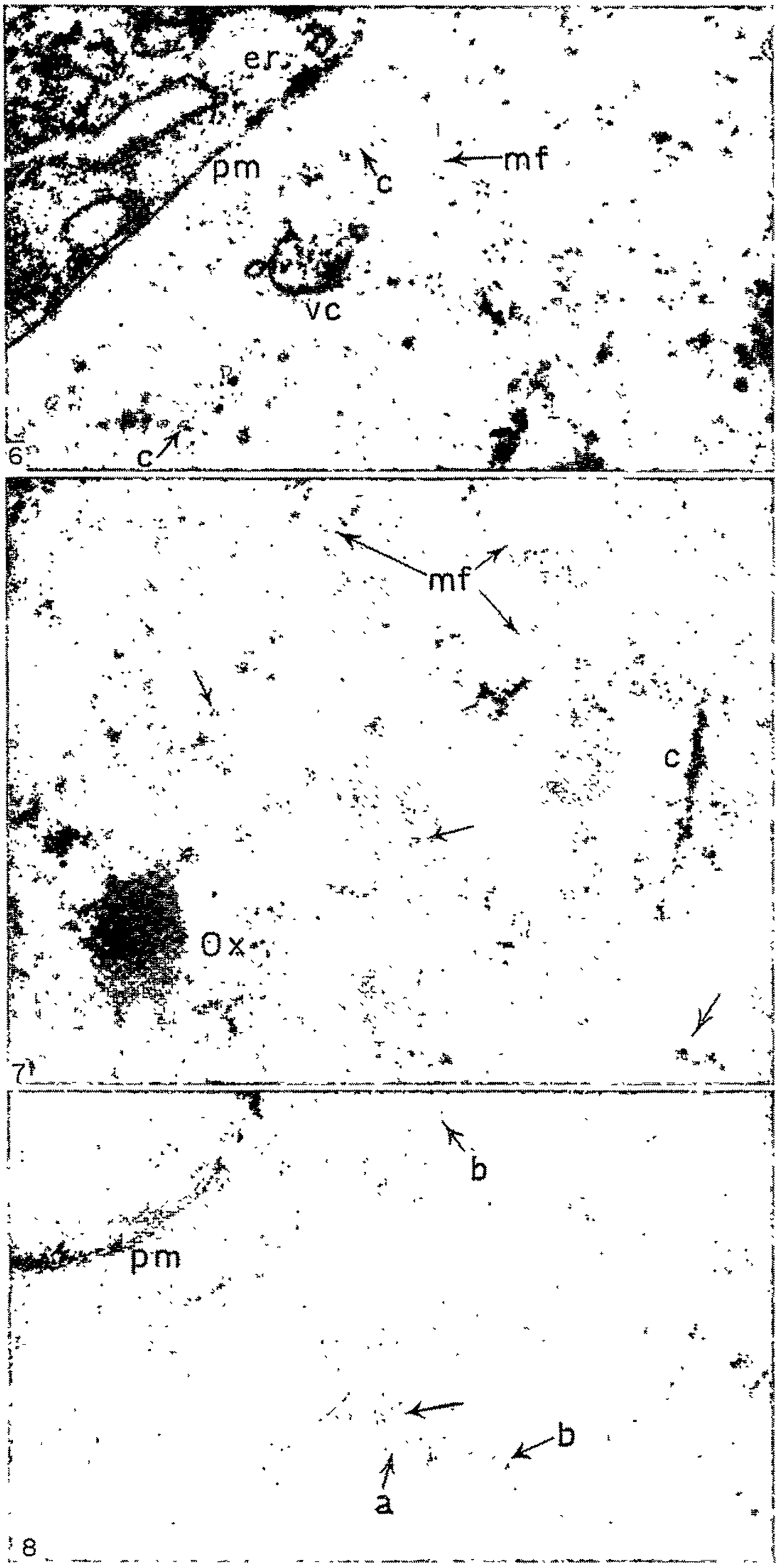


PLATE 3

A.O.B. f.p. 980

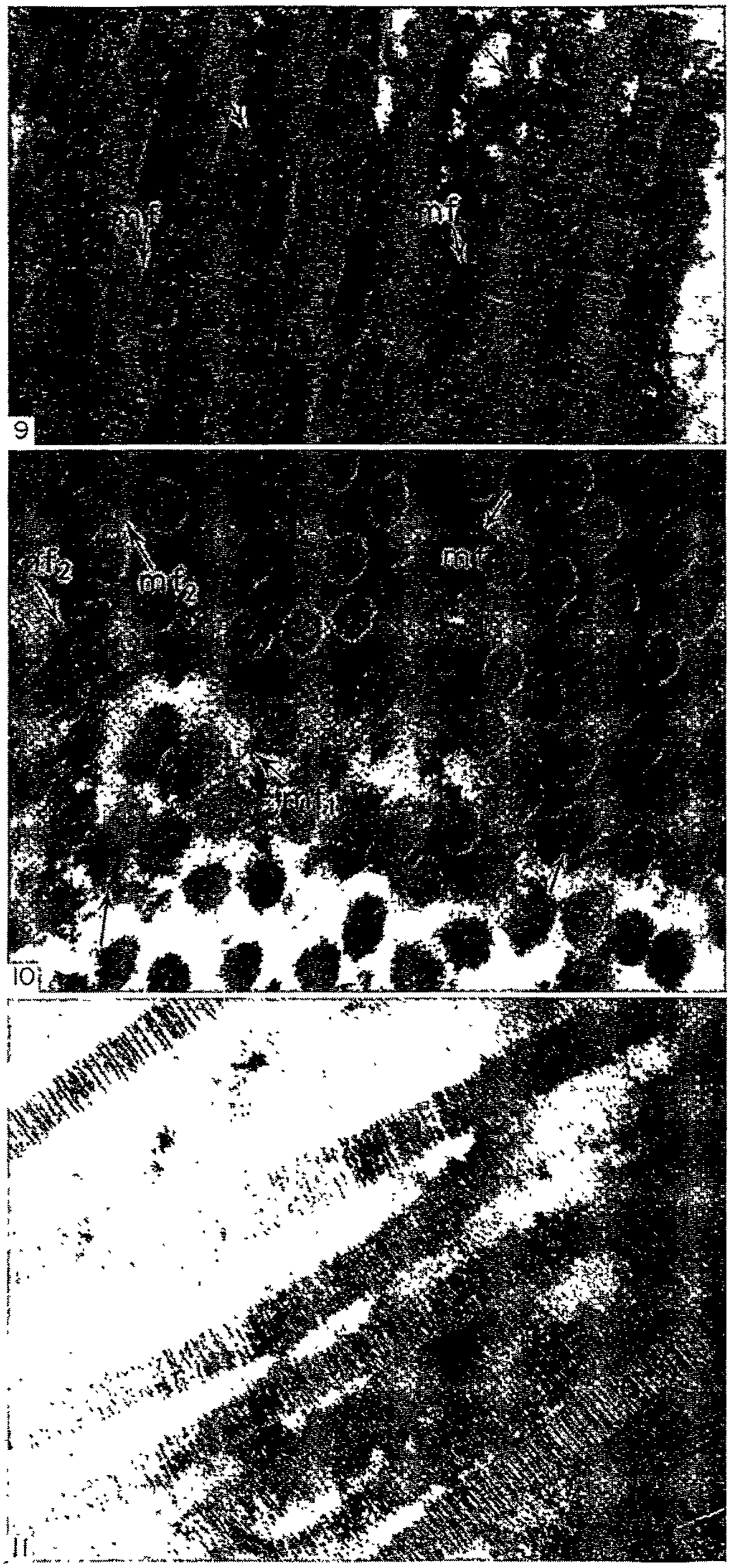


PLATE 4

PLATE 4

FIG. 9. Collagen fibrils 300–350Å diameter. Note the electron density of the fibrils; mf, microfibrils orientated parallel to collagen fibrils; amorphous portion of the microfibril (arrows). Osmium fixation, uranyl acetate. ×145,600.

FIG. 10. Transverse section of collagen fibrils 300–350Å diameter. mf_1, microfibrils, at right angles to collagen fibrils; mf_2, parallel to collagen fibrils; amorphous material associating with collagen fibrils (arrows). Osmium fixation, uranyl acetate. ×145,600.

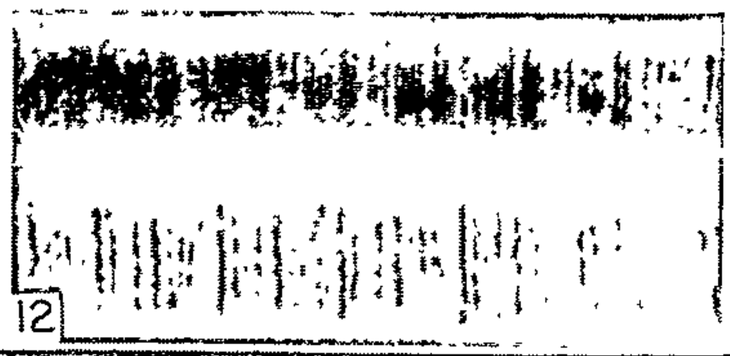
FIG. 11. Collagen fibrils 520Å. Note reduction of intensity of microfibrillar reticulum (*cf.* Fig. 9). Osmium fixation, uranyl acetate. ×145,600.

PLATE 5

FIG. 12. Comparison of young and more mature collagen fibrils. Note marked difference in electron density of the two fibrils. Osmium fixation, uranyl acetate. ×145,600.

FIG. 13. Ox, oxytalan fibril; of, oxytalan micro fibril; beaded elements (arrows); c, collagen fibril. Note, micro fibrils appear to be connected to adjacent micro fibrils by lateral branches. Osmium fixation, uranyl acetate. ×135,800.

FIG. 14. Ox, bundles of oxytalan micro fibrils in transverse section; of_1, oxytalan micro fibril diameter 75–100Å; of_2, 150–175Å. Note amorphous material associated with larger micro fibril. Osmium fixation, uranyl acetate. ×84,700.



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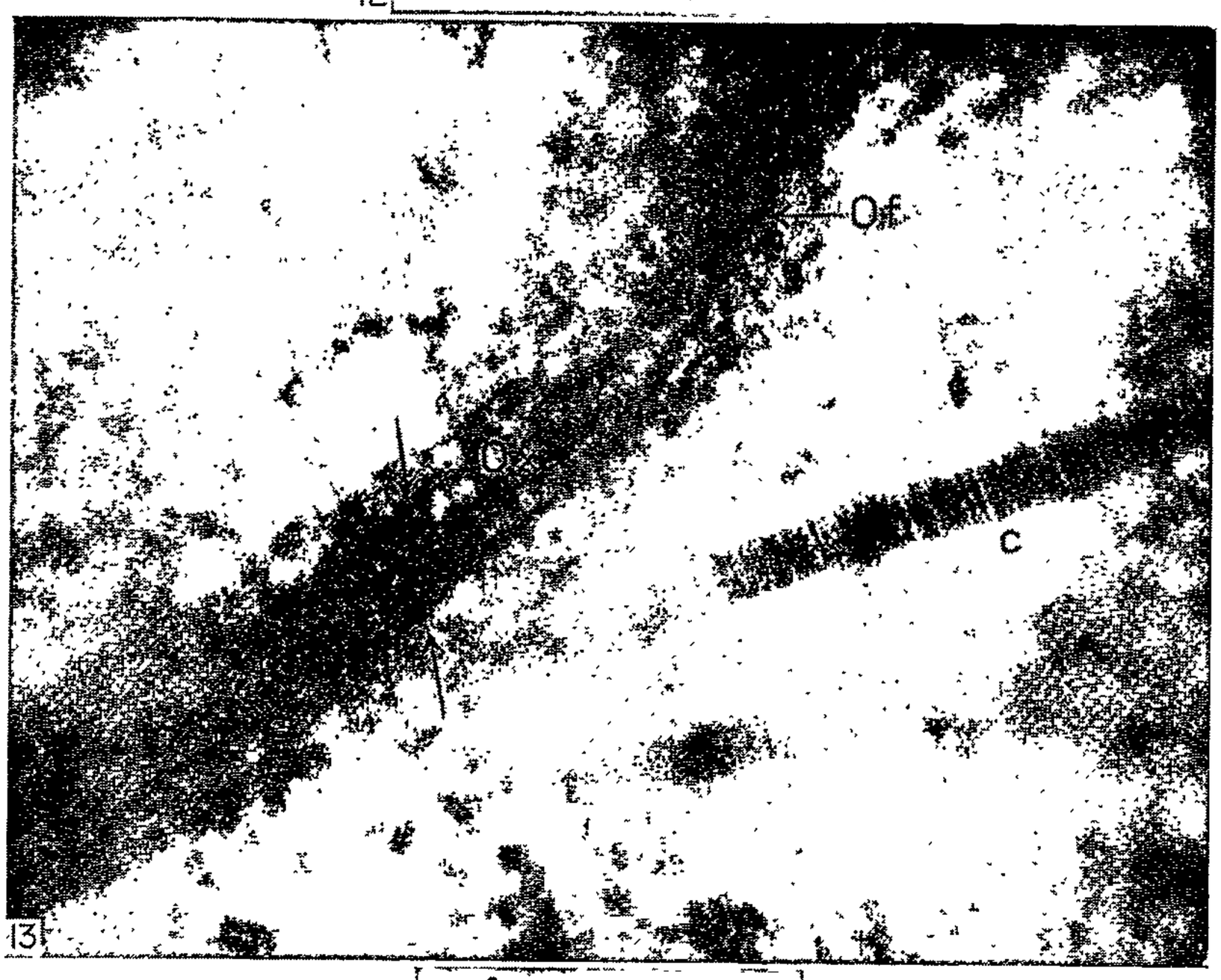

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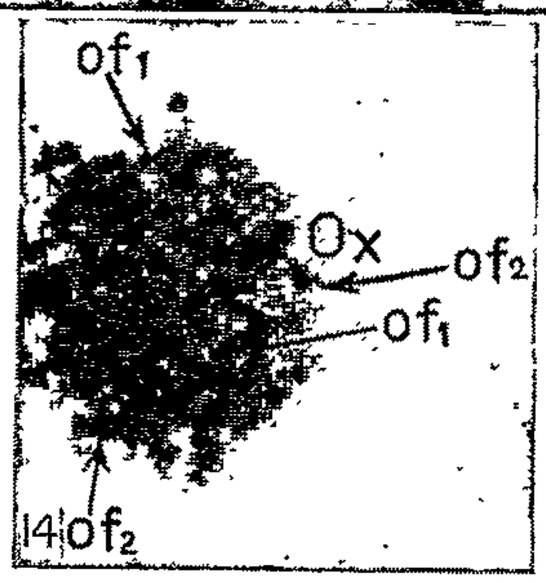

14

PLATE 5

A.O.B. f.p. 982

FIG. 15. Ox, bundles of oxytalan micro fibrils; c, collagen fibrils. Osmium fixation, uranyl acetate. ×46,200.

FIG. 16. Fibroblast and extracellular substance of the developing periodontium. N, nucleus; pm, plasma membrane; cf, collagen fibre; Ox, bundle of oxytalan micro fibrils with a diameter of approximately 3000Å; Ox_1, bundle of oxytalan micro fibrils with a diameter of approximately 1250Å; c, collagen fibrils. Osmium fixation, uranyl acetate. ×10,500.

PLATE 6

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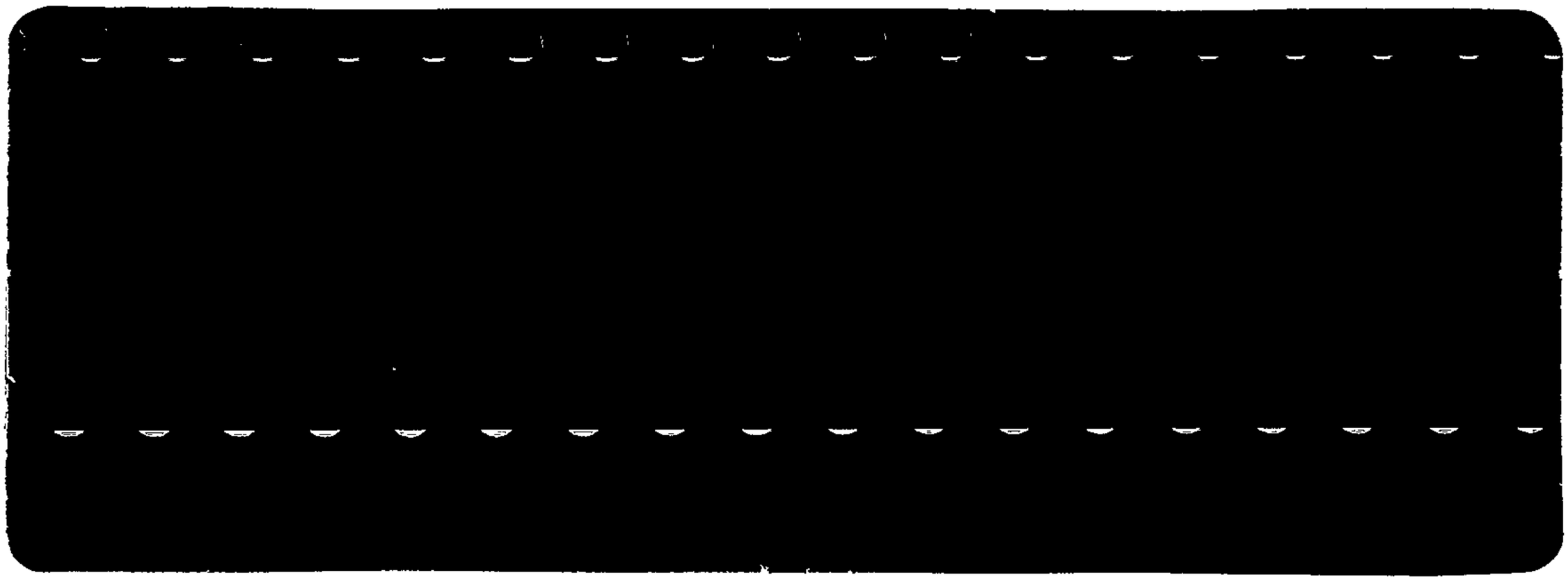

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THE PROTEIN-POLYSACCHARIDE COMPLEX OF THE DEVELOPING HUMAN PERIODONTIUM

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Summary—Material from the developing human periodontium was treated by (1) fixation in glutaraldehyde and stained with uranyl acetate, (2) after fixation, oxidized with peracetic acid and stained with uranyl acetate, (3) as in (2) but β-glucuronidase digestion followed oxidation. Control specimens from (3) were also treated with saccharic acid. Periodontal microfibrils and oxytalan fibrils were partially denatured by peracetic acid oxidation and an amorphous component associated with the microfibrils and the oxytalan fibrils was clearly demonstrated. Both microfibrils and oxytalan fibrils were disrupted by β-glucuronidase after peracetic acid oxidation. The amorphous component appeared to be particularly labile to the enzyme. This indicates that both the microfibrils and oxytalan fibrils are protein-polysaccharide complexes with the amorphous component corresponding to the polysaccharide moiety. Microfibrils appeared to link with, and to have a preferred orientation to, collagen fibrils.

INTRODUCTION

IT HAS been suggested that irregularly beaded microfibrils seen in the developing human dental pulp and developing periodontium correspond to protein-polysaccharide complexes (GRIFFIN and HARRIS, 1967). It was also suggested that these microfibrils by end-to-end linkage and lateral aggregations could form oxytalan fibrils. Appropriate histochemical techniques, originally suggested by FULLMER and LILLIE (1958), have been used to identify the oxytalan fibre as a protein-polysaccharide complex, and FULLMER (1965) distinguished the oxytalan fibre, by reason of its polysaccharide component, from the elastic fibre. CARMICHAEL and FULLMER (1966) described the ultrastructure of the oxytalan fibre in the periodontal membrane of incisors of Sprague Dawley rats. The fibre consisted of bundles of filaments without any apparent structural components joining them. The filaments were about 150–160Å in diameter, about the same distance apart and were without any regular periodicity.

The purpose of this study was to identify the oxytalan fibril and the ground substance microfibril as a protein-polysaccharide complex.

MATERIAL AND METHODS

The material from the human periodontium was procured in the manner previously described (GRIFFIN and HARRIS, 1967) and was fixed in 25 per cent glutaraldehyde in cacodylate buffer, pH 7·4, at 0°C for 4 hr. Some of this material was

immediately embedded and sectioned as below. The following procedures were used for the remainder:

(a) Some specimens were oxidized with peracetic acid at room temperature for 30 min.

(b) Following peracetic acid oxidation, some material was digested with β-glucuronidase (Nutritional Biochemical Corp.; 15 mg in 50 ml of 0·1M acetate buffer, pH 4·5). Some of it was treated with β-glucuronidase inhibitor, saccharic acid, at 0·01–0·1M concentrations and used as a control.

(c) Some material was first digested with β-glucuronidase for 48 hr and then oxidized by peracetic acid oxidation.

In each instance the tissue was dehydrated in increasing concentrations of acetone (25, 50 and 75 per cent for 15 min each) and, after rinsing in three changes of 100 per cent acetone, embedded in Araldite. Polymerization was obtained at 60°C for 24 hr. Thin sections were cut on an L.K.B. ultramicrotome and stained on the grid with uranyl acetate for 1 hr. Electron micrographs were restricted to grey sections.

RESULTS

Extracellular microfibrils and oxytalan fibrils. Glutaraldehyde-fixed material

The ground substance was composed of a reticulum of microfibrils, 30–40Å, in which collagen fibrils were present (Figs. 1 and 2). The diameter of the beaded elements of the microfibrils was approximately 50–60Å and an amorphous material appeared to be associated with them. This reticulum appeared to be intimately associated with collagen fibrils (Fig. 4). Oxytalan fibrils, 1500–2200Å in diameter, were seen to be composed of microfibrils morphologically similar to ground substance microfibrils (Fig. 3). An amorphous substance was also associated with the collagen fibrils.

(a) *Peracetic acid oxidized material.* The ground substance microfibrils were much more conspicuous after the tissue had been oxidized with peracetic acid (Figs. 5 and 6). The fibrils and their beaded elements were larger, having diameters of 40–75Å and 150Å respectively. The amorphous material associated with the beaded elements was much more clearly defined than in the material not oxidized with peracetic acid.

Isolated collagen fibrils and short lengths of striated material seemed to be associated in some areas with the amorphous component of the ground substance microfibril (Fig. 6).

Large oxytalan fibrils, 3900–4700Å, composed of beaded microfibrils 50–75Å in diameter, were present (Figs. 5, 7 and 8). The diameter of the beaded elements was in the range of 150–200Å. In transverse section extensive amorphous material was seen to be associated with the microfibrils (Fig. 5). Oxytalan fibrils were markedly more electron-dense (Figs. 5, 7 and 8) than in the unoxidized material (Fig. 3).

(b) *Peracetic acid oxidized material treated with β-glucuronidase.* The effect of β-glucuronidase, after peracetic acid oxidation of the tissue, was either removal of

the amorphous component associated with the microfibrils (Figs. 9, 10 and 11) leaving microfibrils or remnants of them or, more commonly, there were areas devoid of microfibrils surrounded by what appeared to be clumps of microfibrils (Fig. 12). As compared to control sections (Figs. 13 and 14) there appeared to be a disruption of the ground substance microfibrillar reticulum. No structures which could be identified as oxytalan fibrils could be seen.

(c) *β-Glucuronidase-digested, peracetic acid-oxidized material.* The results of this treatment were similar to those in which an enzyme-inhibitor (saccharic acid) was added and to those in which peracetic acid oxidation alone followed fixation.

DISCUSSION

The results indicate that, when the tissue is pretreated with peracetic acid, β-glucuronidase disrupts the ground substance microfibrillar reticulum and oxytalan fibrils cannot be identified. There is usually marked clumping of the ground substance elements. This suggests that ground substance microfibrils and oxytalan microfibrils are similar in nature. The effect of the enzyme probably is to cleave a glucuronidase linkage, so making end groups available which results in clumping of the microfibrils. The amorphous component is possibly removed by this procedure or alternatively is incorporated in the clumped material. Nevertheless we cannot exclude the possibility that the enzymes used were contaminated with proteinases, which could in certain cases remove all the ground substance elements.

The results indicate that, because the ground substance microfibrillar reticulum is disrupted by β-glucuronidase, it is a protein-polysaccharide complex.

It is therefore suggested that the fibrillar component of the reticulum corresponds to the protein moiety and that the amorphous component corresponds to the polysaccharide moiety, whilst the beaded component could represent a globular linkage protein. A similar structure has been postulated by FITTON JACKSON (1964).

The amorphous component of the microfibrillar reticulum and of the oxytalan fibril is apparently unmasked by peracetic acid oxidation and both structures stain rather intensely with uranyl acetate. FULLMER and LILLIE (1958) explained the staining of oxytalan fibres with aldehyde fuchsin after peracetic acid oxidation on the basis that bonds in the polysaccharide moiety were opened by this procedure so that it then accepted aldehyde fuchsin. A similar explanation might possibly apply to ground substance microfibrils and oxytalan fibrils as observed in this study, namely that peracetic acid oxidation opened up bonds in the polysaccharide moieties of both the oxytalan fibril and the ground substance microfibril so that they then accepted uranyl ions.

The diameters of the oxytalan fibrils and ground substance microfibrils were larger in material oxidized with peracetic acid than in untreated material. This is a further indication of similarity between ground substance microfibrils and the oxytalan fibrils. Perhaps the swelling of both these structures indicates that some denaturation of the proteinaceous component has occurred.

The apparent similarity in the reaction of the microfibrils and oxytalan fibrils

suggests that they are of the same nature and that the oxytalan fibril could form as a result of end-to-end linkage and later aggregation of ground substance microfibrils.

MATHEWS (1965), by electrophoresis, demonstrated protein–polysaccharide complexes associated with collagen fibrils and suggested that this complex would vary from tissue to tissue and from species to species and that self aggregation of these complexes could occur. It is possible that the periodontal microfibrillar reticulum is of a similar nature except in respect to a linkage protein (beaded element) being interposed between the protein core and the polysaccharide moiety and also as regards its orientation to collagen fibrils (GRIFFIN and HARRIS, 1967). Short lengths of striated material, presumably young collagen, were seen to be associated with the amorphous components of the ground substance microfibril, suggesting that this component could be a site of nucleation for precipitation of collagen in a fibrous form. WOOD (1964) has postulated such a phenomenon.

Collagen fibrils aggregated to form collagen fibres have branches of the microfibrillar reticulum preferably orientated at right angles to them and parallel to their long axes (GRIFFIN and HARRIS, 1967). Similar orientation was seen in the glutaraldehyde-fixed material and the amorphous component of the microfibril was seen to associate with the collagen fibrils.

Acknowledgement—We wish to thank Professor K. W. CLELAND, Department of Histology and Embryology, University of Sydney, especially in regard to the high resolution electron microscopy.

Résumé—Du parodonte humain, en voie de développement est (1) fixé au glutaraldéhyde et coloré à l'acétate d'uranyle, (2) oxydé, après fixation, avec l'acide périacétique et coloré à l'acétate d'uranyle (3) traité comme en (2), en faisant suivre l'oxydation par une digestion à l'aide de β-glucuronidase. Des pièces témoins de (3) sont traitées par l'acide saccharique.

Des microfibrilles ligamentaires et des fibrilles oxytalanes sont partiellement dénaturées par oxydation à l'acide périacétique et un composé amorphe, lié aux microfibrilles et aux fibrilles oxytalanes, est nettement mis en évidence. Ces Microfibrilles et fibrilles oxytalanes sont détruites par la β-glucuronidase, après oxydation à l'acide périacétique. Le composé amorphe est particulièrement sensible à l'enzyme. Il semble que les microfibrilles et les fibrilles oxytalanes soient constituées par des complexes protéino-polysacchardiques, le composé amorphe correspondant à la fraction polysaccaridique. Les microfibrilles ont une orientation préférentielle et, semblent en rapport avec les fibrilles collagènes.

Zusammenfassung—Substanz aus sich entwickelnden menschlichen Periodontien wurde behandelt: (1) durch Fixierung in Glutaraldehyd und Färbung mit Uranylacetat, (2) nach Fixierung durch Oxydierung mit Peressigsäure und Färbung mit Uranylacetat, (3) durch Behandlung wie bei (2), wobei nach der Oxydation β-Glukuronidase einwirkte. Kontrollschnitte aus der Gruppe (3) wurden auch mit Zuckersäure behandelt. Parodontale Mikrofibrillen und Oxytalan-Fibrillen wurden durch Oxydation mit Peressigsäure teilweise denaturiert, und es wurde eindeutig eine amorphe Komponente dargestellt, die mit den Mikrofibrillen und den Oxytalanfasern zusammenhängt. Sowohl Mikrofibrillen als auch Oxytalanfasern wurden nach Oxydation mit Peressigsäure durch β-Glukuronidase zerstört. Die amorphe Komponente schien gegenüber dem Enzym besonders empfindlich zu sein. Dies weist darauf hin, daß sowhol Mikrofibrillen als auch Oxytalanfasern Protein-Polysaccharid-Komplexe mit der dem

Polysaccharid-Anteil entsprechenden amorphen Komponente sind. Die Mikrofibrillen schienen sich mit den Kollagenfibrillen zu verbinden und sich nach ihnen vorzugsweise zu orientieren.

REFERENCES

CARMICHAEL, G. G. and FULLMER, H. M. 1966. The fine structure of the oxytalan fiber. *J. Cell Biol.* **28,** 33–36.

FULLMER, H. M. 1965. The histochemistry of the connective tissues. *Int. Rev. connect. Tissue Res.* **3,** 34.

FULLMER, H. M. and LILLIE, R. D. 1958. The oxytalan fiber, a previously undescribed connective tissue fiber. *J. Histochem. Cytochem.* **6,** 425–430.

GRIFFIN, C. J. and HARRIS, R. 1967. The fine structure of the developing human periodontium. *Archs. oral Biol.* (In press).

MATHEWS, M. B. 1965. The interaction of collagen and acid mucopolysaccharides. *Biochem. J.* **96,** 710–716.

WOOD, G. C. 1964. The precipitation of collagen fibres. *Int. Rev. connect. Tissue Res.* **2,** 1–28.

PLATES 1–4 OVERLEAF

PLATE 1

FIG. 1. Extracellular substance of the developing human periodontium. c = collagen fibrils; mf = ground substance microfibrils. Glutaraldehyde-fixed, uranyl acetate. ×60,000.

FIG. 2. Microfibrillar reticulum of developing periodontium. mf = microfibril; a = beaded elements; arrows = amorphous component. Glutaraldehyde- fixed, uranyl acetate. ×189,600.

FIG. 3. Ox = oxytalan fibrils; c = bundles of collagen fibrils aggregated to form a collagen fibre. Glutaraldehyde-fixed, uranyl acetate. ×64,600.

FIG. 4. c = collagen fibril; mf = microfibrils orientated at right angles to transversely sectioned collagen; arrows = amorphous component. Glutaraldehyde-fixed, uranyl acetate. ×40,000.

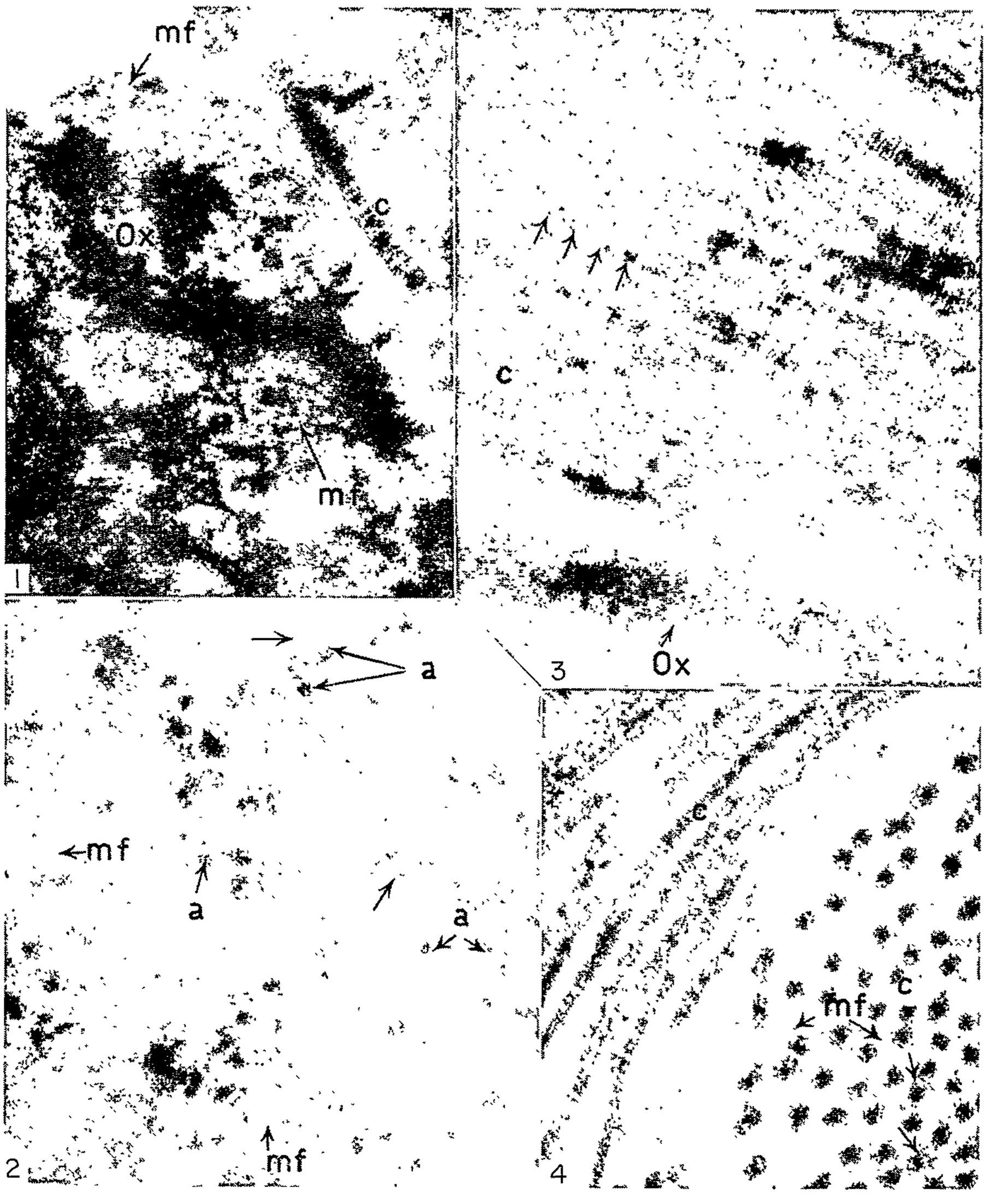

PLATE 1

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PLATE 2

PLATE 2

FIG. 5. Ox = oxytalan fibrils; mf = ground substance microfibrils; a = beaded elements; arrows = amorphous component. Glutaraldehyde-fixed, peracetic acid-oxidized, uranyl acetate. ×60,000.

FIG. 6. c = collagen fibril; mf = ground substance microfibril; a = beaded element; arrows = amorphous component; striated material, associated with amorphous substance, S, with collagen fibril, S_1 (*cf.* FIG. 2). Glutaraldehyde-fixed, peracetic acid-oxidized, uranyl acetate. ×149,300.

FIG. 7. Ox = oxytalan fibrils; c = collagen fibrils. Glutaraldehyde-fixed, peracetic acid-oxidized, uranyl acetate. ×64,600

FIG. 8. Ox = oxytalan fibrils. Glutaraldehyde-fixed, peracetic acid-oxidized, uranyl acetate. ×64,600.

B

PLATE 3

FIG. 9. arrow = remnants of ground-substance microfibrils; e = clumping of ground-substance elements. Glutaraldehyde-fixed, peracetic acid-oxidized, β-glucuronidase, uranyl acetate. ×23,000.

FIG. 10. Collagen fibrils, c, and ground-substance of the periodontium. Beaded microfibrils (arrows) are present and there is an absence of the amorphous component. The collagen fibrils are markedly less electron-dense. Glutaraldehyde fixation, peracetic acid-oxidized, β-glucuronidase, uranyl acetate. ×26,600.

FIG. 11. Remnants of ground-substance elements and clumping. mf = microfibrils; e = clumped material (*cf.* FIG. 6). Glutaraldehyde-fixed, peracetic acid-oxidized, β-glucuronidase. ×149,300.

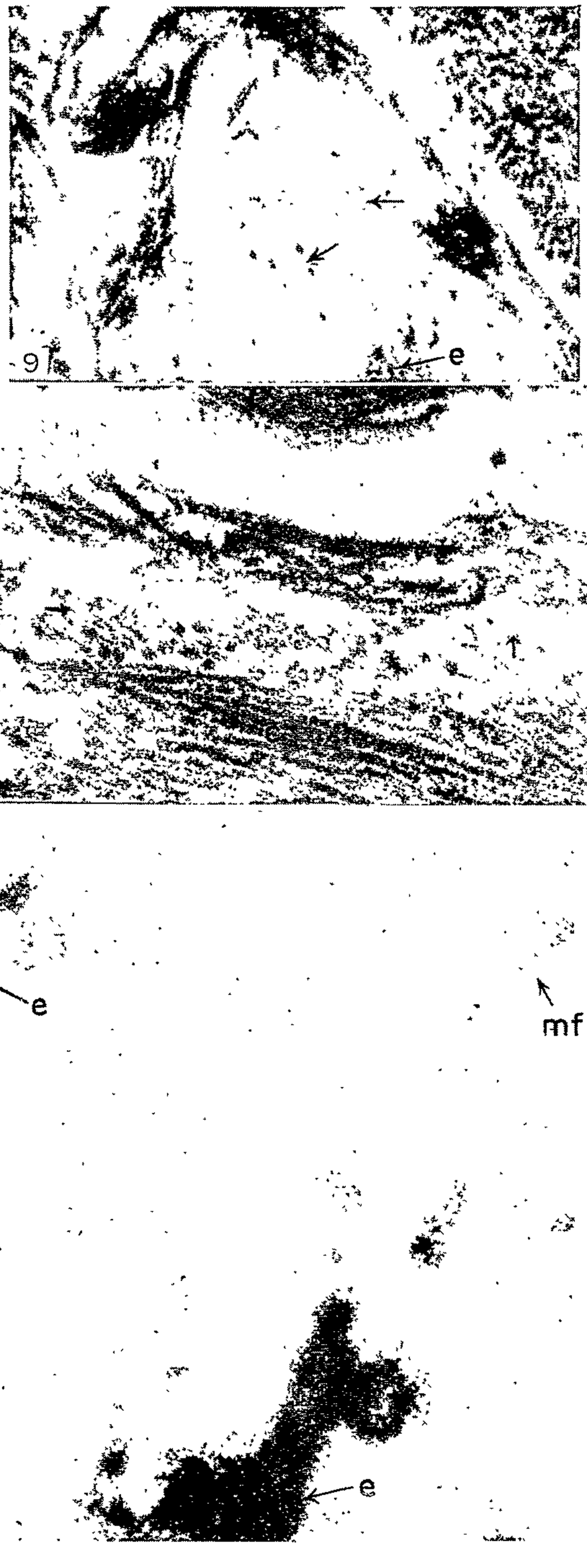

PLATE 3

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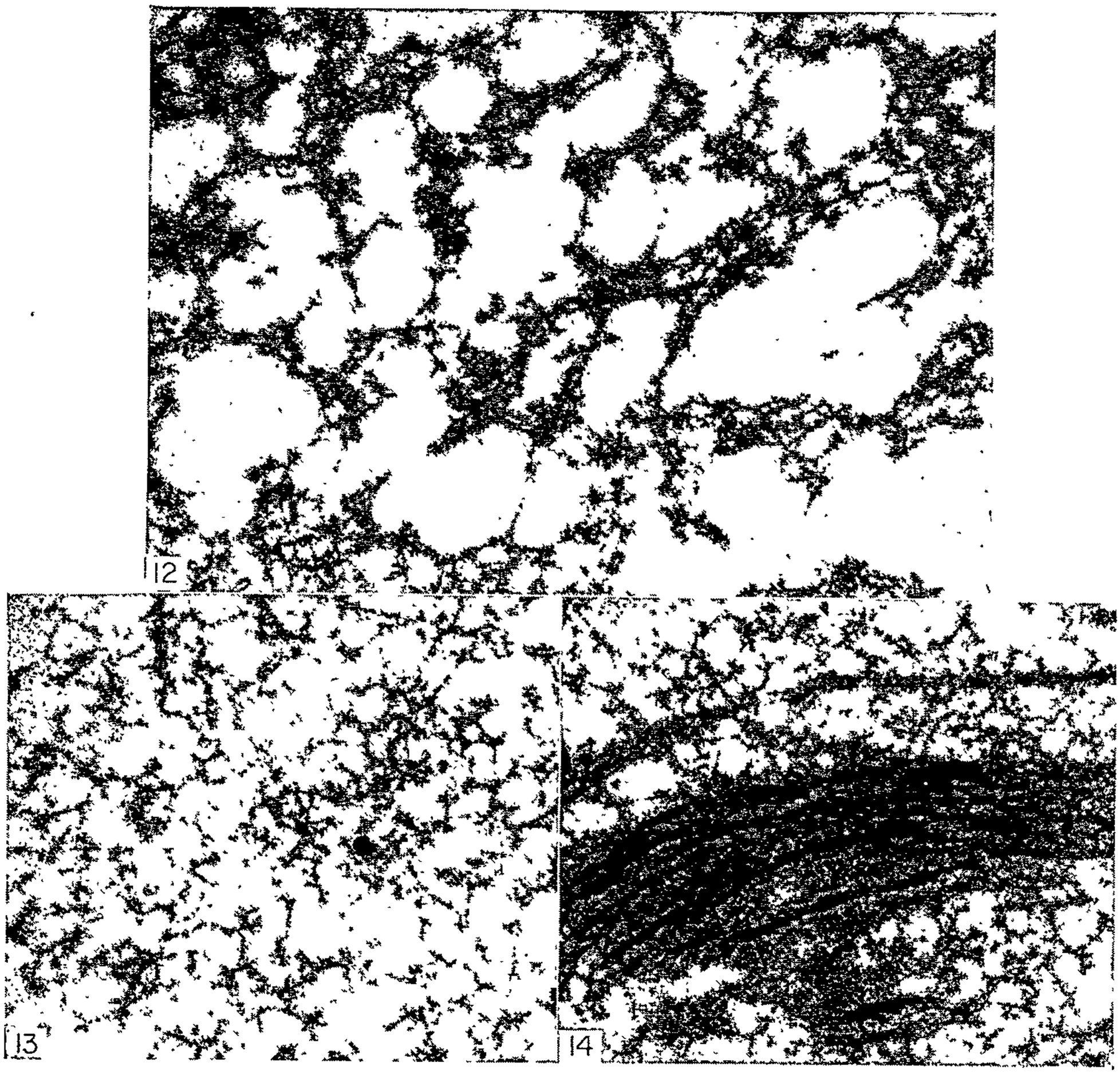

FIG. 12. Clumping of periodontal ground-substance elements. Glutaraldehyde fixation, peracetic acid oxidized, β-glucuronidase, uranyl acetate. $\times 10{,}000$.

FIG. 13. Ground-substance of the periodontium with microfibrils forming a reticulum. Control section. $\times 23{,}000$.

FIG. 14. Ground substance of the periodontium showing collagen fibrils markedly electron-dense (*cf.* FIG. 10). Control section. $\times 23{,}000$.

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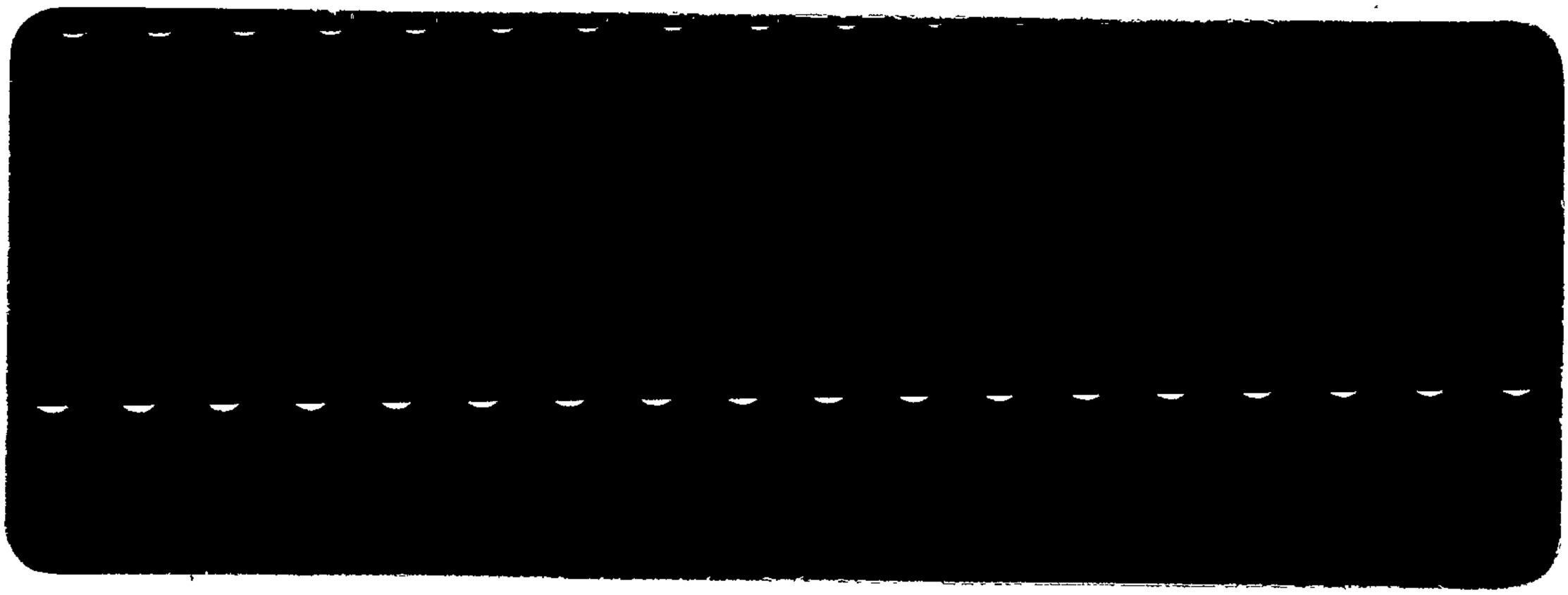

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UNMYELINATED NERVE ENDINGS IN THE PERIODONTAL MEMBRANE OF HUMAN TEETH

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Summary—Electron micrographs of tissue from developing and functional human periodontium from twelve teeth show the presence of unmyelinated and myelinated nerve fibres. The presence of vesicles and small mitochondria in the axons of the unmyelinated fibres suggests that these are nerve endings. The myelinated nerve fibres are of uniform fine diameter corresponding to the Group III category. They are seen to terminate, after losing their myelin lamellae, as axonal swellings covered by basement membrane and exposed to the ground substance. Electron-dense bodies, vesicles and mitochondria are present.

INTRODUCTION

NERVE endings originating from large myelinated nerve fibres have been identified in the human periodontium (VAN DER SPRENKEL, 1936; LEWINSKY and STEWART, 1937). LEWINSKY and STEWART (1937) described these nerve endings as spindle shaped, whilst VAN DER SPRENKEL (1936) referred to them as "end rings" and the latter suggested that they might influence chewing pressure. These nerve endings were said to arise from myelinated nerve fibres larger than 10 μ in diameter (BRASHEAR, 1936).

KEREBEL (1964) noted the rich innervation of the periodontal membrane, in contrast to that of the pulp in the kitten, as is the case with the innervation of the periodontium of the adult cat (LEWINSKY and STEWART, 1937). In the periodontium the average size of the nerve fibres is much greater than in the dental papilla. KEREBEL (1964) further noted the extreme variety of the periodontal innervation and the presence of unmyelinated fibres in relation to blood vessels. He was unable to demonstrate end organs in the periodontal tissues of the new-born cat.

SIMPSON (1966) described thick parallel nerve bundles which ran parallel to the long axis of the tooth and gave off branches to form plexuses and bundles of fibres. These latter fibres travelled considerable distances and then looped back upon themselves in a complicated pattern. Some fibres terminated in free unmyelinated extremities and others joined to form a network. Other unmyelinated nerve fibres occasionally ended in knob-like enlargements, whilst others broke up into irregularly branching endings of variable form.

BERNICK (1964) has described both myelinated and unmyelinated fibres in the periodontal membrane of molar teeth of the guinea pig.

LAUTENBACH (1966) has described nerve fibres in inflamed and normal gingival tissues of humans and demonstrated swellings on some of the larger fibres and the twisting and branching of the fine fibres.

The present paper deals with the fine structure of free unmyelinated nerve endings in the periodontal ligament in both developing and functional human periodontium.

MATERIAL AND METHODS

(1) The material examined consisted of portions of the dental sac surrounding unerupted third molar teeth extracted under general anaesthesia from four patients aged 14–16 yr. Radiographically, all the teeth had reached the end of the crown formation stage and root formation was commencing. The material was obtained as previously described (GRIFFIN and HARRIS, 1967) and placed in PALADE's (1952) fixative within one minute of removal. The tissue was cut into 0·5 × 1·0 mm pieces and placed in fresh PALADE's (1952) osmium tetroxide fixative at 0°C for 4 hr. After fixation, the pieces were dehydrated in increasing concentrations of acetone (25, 50 and 75 per cent for 15 min each) and after rinsing in three changes of acetone were embedded in Araldite. Polymerization was effected at 60°C for 24 hr.

(2) Pieces of periodontium attached to eight freshly extracted premolar teeth of a male patient aged 11 yr and a female patient aged 13 yr were immediately covered with PALADE's (1952) osmium tetroxide fixative. Whilst immersed in the fixative the soft tissue was removed from the tooth surface and cut into pieces approximately 1 × 1 mm. The further treatment was as described above. Sections 4 μ thick were cut, stained with toluidine blue and examined. Sections were cut at 500 Å from both sets of tissue on an L.K.B. ultramicrotome and stained on the grid with uranyl acetate for 1 hr. Sections of the functional tissues were examined at approximately 2000 Å intervals. Electron micrographs and observations were made on Hitachi H.S.7 and Hitachi HU-11B electron microscopes.

OBSERVATIONS

(1) *Developing periodontium*

Schwann cells could be recognized by the presence of a characteristic plasma membrane and the axons of unmyelinated nerve fibres (Figs. 1 and 2). The nuclei of these cells were seen to have a marginal condensation of chromatin and several nucleoli. The Golgi complex was usually juxtanuclear (Fig. 1) and a few channels and dilated profiles of the rough-surfaced endoplasmic reticulum and free ribosomes were seen (Fig. 4). Mitochondria were numerous and small, 3000–4000 Å in diameter, and had regular cristae (Fig. 2). The cytoplasm contained intracytoplasmic filaments about 60 Å in diameter (Fig. 4) and microtubules about 200 Å in diameter (Fig. 1). Membrane-bounded bodies containing electron-dense material, possibly lysosomes, were also seen (Fig. 1). The plasma membrane of the Schwann cell was approximately 75 Å thick and the amorphous basement membrane was about 500 Å thick (Figs. 1, 2, 3 and 4). What appeared to be a cilium was seen in one Schwann cell (Fig. 1). It arose deep in the cell, near the Golgi complex, and projected in the direction of the plasma membrane. It was seen to contain double axial filaments and the width of the shaft was about 3000 Å whilst its length was at least 20,000 Å.

Unmyelinated nerve endings. Unmyelinated nerve fibres were seen to be surrounded by the plasma membrane of the Schwann cell (Figs. 1, 2, 3 and 4). The plasma membrane of these axons was usually separated from the plasma membrane of the Schwann cell by an amorphous substance (Figs. 1 and 4). The axons of the unmyelinated nerve fibres contained elements which suggested that they corresponded to nerve endings. The elements within the axons were small mitochondria and vesicles 400–1000 Å in diameter (Figs. 1, 3 and 4).

The axons also contained filaments about 60 Å in diameter and microtubules about 200 Å in diameter (Figs. 1 and 4).

Twenty-five unmyelinated nerve fibres were measured and found to have a mean diameter of 7192 Å (S.D. 2880 Å).

(2) *Functional periodontium*

Unmyelinated nerve fibres. In sections at 2000 Å intervals, unmyelinated nerve fibres were seen closely associated with myelinated nerve fibres in the functional periodontium. Some of these nerve fibres were surrounded by the plasma membrane of the Schwann cell whilst in others the plasma membrane of the axon was exposed to the ground substance (Figs. 5–9). When exposed to the ground substance the plasma membrane of the axon was surrounded by a basement membrane and collagen fibrils (Figs. 6, 8 and 9).

The exposed axons contained elements which suggest they correspond with nerve endings and were similar to those seen in the developing periodontium. The diameters of the nerve endings ranged between 1000 and 6000 Å.

The mean diameter of 122 unmyelinated nerve fibres in functional periodontium was found to be 4158 Å (S.D. 2423 Å). The mean diameter of the 147 fibres in functional and developing periodontium was found to be 4630 Å (S.D. 2872 Å) and a histogram of the distribution shows peaks between 1000 and 3000 Å and between 4000 and 6000 Å (Fig. 10).

Myelinated nerve fibres. Myelinated nerve fibres, corresponding to Group III fine fibres, were traced to their endings in sections at 2000 Å intervals. (Group III or A

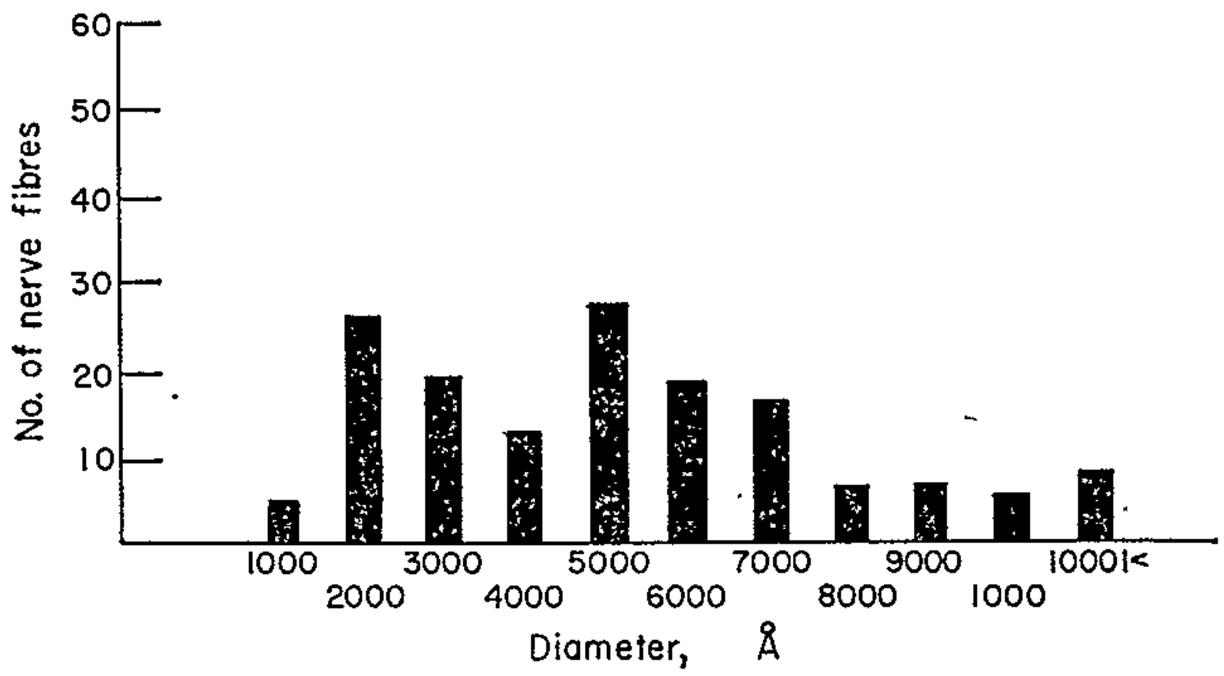


FIG. 10. Distribution of the diameters of 147 unmyelinated nerve fibres in the developing and functional human periodontium.

delta fibres are myelinated nerve fibres ranging in diameter between 1–6 μ and conducting at the rate of 5–30 m/sec. (LLOYD, 1943.) After losing the myelin sheath, the fibre divided into several branches of uniform diameter (Fig. 11) which were surrounded by Schwann cell cytoplasm. They were seen to terminate as several axonal swellings approximately 0·5–1·5 μ in diameter covered by basement membrane exposed to the ground substance, and they contained numerous vesicles, vacuoles, electron-dense bodies and mitochondria (Figs. 12, 13 and 14). Matrices of many mitochondria appeared to be particularly dense (Figs. 4, 13 and 14).

DISCUSSION

Schwann cells

Human periodontal Schwann cells are very similar to the Schwann cells described in the splenic nerve of the cat by ELFVIN (1958) and are completely enveloped by a basement membrane. Microtubules were seen in the cytoplasm, in some instances aggregated at the plasma membrane (Fig. 1), but they do not appear to achieve the complexity described in the shrimp (*Penaeus japonicus*) by HAMA (1966). However, the disclosure of the true distribution of these elements may require the special fixation and staining methods described by SANDBORN, *et al.* (1964) and these authors pointed out that microtubules are not exclusive to neurones. A structure resembling a cilium was seen in one Schwann cell (Fig. 1). According to GRILLO and PALAY (1963), it is quite possible that every Schwann cell in the autonomic nervous system of the adult rat has a cilium. These authors suggested that because ciliated Schwann cells appear only in the sheaths of unmyelinated nerve fibres they reflect a profound change in the nature of the Schwann cell in respect of its progressive differentiation. However, a feature of the cilia in these rat Schwann cells was the presence of a central tubular or cylindrical structure about 900 Å in diameter. On the other hand, the structure identified as a cilium in human periodontal Schwann cells appeared to have a two-filament axial form, and thus corresponds to the usual structure of cilia (FAWCETT, 1961).

The plasma membrane of the Schwann cell was seen to be separated from the plasma membrane of the axon of unmyelinated nerve fibres by an amorphous substance (Figs. 1 and 4). This material has been interpreted by ROBERTSON (1956) as representing an extension of the extracellular substance.

Unmyelinated nerve endings

Unmyelinated nerve endings, apparently not related to blood vessels, were seen in both functional and developing periodontium (Figs. 1–9). That these fibres represent the nerve endings is suggested by the presence of microvesicles and small mitochondria (BISCOE and STEHBENS, 1966). The diameters of the vesicles correspond to the diameters of synaptic vesicles described in synapses in the central nervous system (GRAY and GUILLERY, 1966). In certain instances the axons covered by basement membranes were exposed to the ground substance (Fig. 4). Sections at 2000 Å intervals suggested that these nerve endings were not derived from myelinated nerve fibres although this cannot be definitely excluded. It would seem, therefore, that these

are nerve endings of the trigeminal nerve which correspond morphologically to dorsal root 'C' fibres or Group IV fibres of the spinal nerves (GASSER, 1941).

Nerve endings of Group III fibres

The terminals of the Group III fibres differed from similar fibres observed in the dental pulp in that they were of uniform diameter (Fig. 11) and did not exhibit the varicosities and constrictions of the latter. The terminal axonal swellings containing vesicles resembling synaptic vesicles, however, were essentially similar to those seen in the dental pulp (HARRIS and GRIFFIN, 1968). No synaptic junctions were observed. In addition there were electron-dense bodies and electron-dense mitochondria. Certain of these electron-dense bodies have been thought to represent accumulations of lipid material. MUNGER (1965) found, in the intraepidermal nerve endings of the snout of the opossum, numerous mitochondria, myelin figures, vesicles, vacuoles and dense lipid bodies. Electron-dense bodies have also been observed in Meissner's corpuscle (CAUNA and ROSS, 1960). These authors interpreted the dense granular and vesicular substance in these nerve endings as representing the final stages in the disintegration of mitochondria. Lipid droplets have also been observed in encapsulated nerve endings in the rat penis (PATRIZI and MUNGER, 1965). It is possible that the functional state of the end organ determines the mitochondrial and vesicular population of the nerve ending.

Résumé—L'étude en microscopie électronique du parodonte humain adulte et en voie de développement de douze dents montre la présence de nerfs myélinisés et amyélinisés. La présence de vésicules et petites mitochondries dans l'axe des fibres amyélinisées semble indiquer qu'il s'agit là de terminaisons nerveuses. Les fibres nerveuses myélinisées sont de diamètre uniforme fin, correspondant au groupe III. Ils se terminent sous la forme de renflements axoniques, recouverts par une membrane basale et s'étendant librement dans la substance fondamentale, après avoir perdu leurs lamelles de myéline. On y note la présence de corps denses, de vésicules ainsi que de mitochondries.

Zusammenfassung—Elektronenmikroskopische Aufnahmen vom sich entwickelnden und vom funktionell ausgebildeten Desmodont von zwölf menschlichen Zähnen zeigen das Vorhandensein markloser und markhaltiger Nervenfasern. Die Existenz von Bläschen und kleinen Mitochondrien in den Axonen der marklosen Fasern legt die Deutung nahe, daß es sich dabei um Nervenendigungen handelt. Die markhaltigen Nervenfasern weisen einen einheitlich kleinen Durchmesser entsprechend der Kategorie der Gruppe III auf. Sie enden nach Verlust ihrer Markscheide als Axonverdickungen, die mit einer Basalmembran bedeckt und in die Grundsubstanz eingelagert sind. Elektronendichte Körper, Bläschen und Mitochondrien sind vorhanden.

REFERENCES

BERNICK, S. 1964. Blood supply and innervation of the teeth, periodontium and temporo-mandibular joint of the guinea pig. *J. dent. Res.* **43**, 812, Suppl. (Abst.).

BISCOE, T. J. and STEHBENS, W. E. 1966. Ultrastructure of the carotid body. *J. cell Biol.* **30**, 563–578.

BRASHEAR, A. D. 1936. The innervation of the teeth. *J. comp. Neurol.* **64**, 169–185.

CAUNA, N. and ROSS, L. L. 1960. The fine structure of Meissner's touch corpuscles of the human fingers. *J. biophys. biochem. Cytol.* **8**, 467–482.

ELFVIN, L.-G. 1958. The ultrastructure of unmyelinated nerve fibres in the splenic nerve of the cat. *J. ultrastruct. Res.* **1**, 428–454.

FAWCETT, D. 1961. Cilia and flagella. In: *The Cell* (Edited by BRACHET, J. and MIRSKY, A. E.) Vol. 2, p. 217. Academic, New York.

GASSER, H. S. 1941. The classification of nerve fibers. *Ohio J. Sci.* **41,** 145–159.

GRAY, E. G. and GUILLERY, R. W. 1966. Synaptic morphology in the normal and degenerating nervous system. In: *International Review of Cytology* (Edited by BOURNE, G. H. and DANIELLI, J. F.) **19,** 111–182. Academic, New York.

GRIFFIN, C. J. and HARRIS, R. 1967. The fine structure of the developing human periodontium. *Archs oral Biol.* **12,** 971–982.

GRILLO, M. A. and PALAY, S. L. 1963. Ciliated Schwann cells in the autonomic nervous system of the adult rat. *J. cell Biol.* **16,** 430–436.

HAMA, K. 1966. The fine structure of the Schwann cell sheath of the nerve fiber in the shrimp (*Penaeus japonicus*). *J. cell Biol.* **31,** 624–632.

HARRIS, R. and GRIFFIN, C. J. 1968. Fine structure of nerve endings in the human dental pulp. *Archs oral Biol.* **7,** 773–778.

KEREBEL, B. 1964. Organisation de l'innervation dentaire et péridentaire à la naissance. *Rev. Belge med. and dent.* **19,** 169–190.

LAUTENBACH, E. 1966. Über Nervstrukturen im Weichgewebe des marginalen Parodonts. *Schweiz. Monatsschr. für Zahnheilk.* **76,** 165–170.

LEWINSKY, W. and STEWART, D. 1937. The innervation of the periodontal membrane of the cat, with some observations on the functions of the end-organs found in that structure. *J. Anat.* **71,** 232–235.

LLOYD, D. P. C. 1943. Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat. *J. Neurophysiol.* **6,** 293–315.

MUNGER, B. L. 1965. The intraepidermal innervation of the snout skin of the opossum. A light and electron microscope study, with observations on the nature of Merkel's *Tastzellen. J. cell Biol.* **26,** 79 97.

PALADE, G. E. 1952. A study of fixation for electron microscopy. *J. exp. Med.* **95,** 285–298.

PATRIZI, G. and MUNGER, B. L. 1965. The cytology of encapsulated nerve endings in the rat penis. *J. ultrastruct. Res.* **13,** 500–515.

ROBERTSON, J. D. 1956. The ultrastructure of a reptilian myoneural junction. *J. biophys. biochem. Cytol.* **2,** 381–393.

SANDBORN, E., KOEN, P. F., MCNABB, J. D. and MOORE, G. 1964. Cytoplasmic microtubules in mammalian cells. *J. ultrastruct. Res.* **11,** 123–138.

SIMPSON, H. E. 1966. The innervation of the periodontal membrane as observed by the apoxestic technique. *J. periodont.* **37,** 374–376.

VAN DER SPRENKEL, H. 1936. Microscopical investigation of the innervation of the tooth and its surroundings. *J. Anat.* **70,** 233–241.

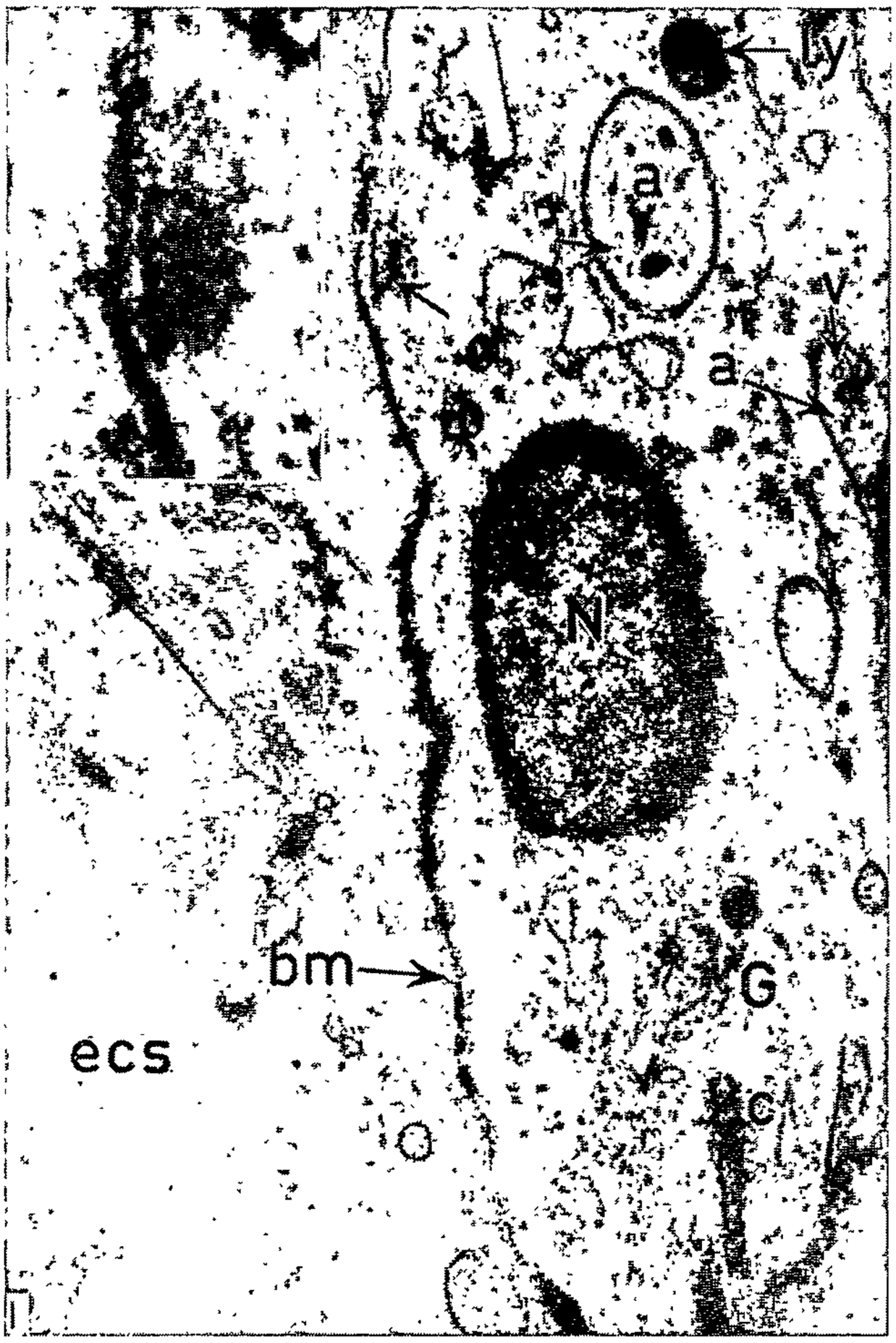


FIG. 1. Schwann cell of developing human periodontium. N, nucleus; G, Golgi apparatus; a, axon; v, synaptic vesicles; ly, electron-dense membrane bounded body; bm, basement membrane; ecs, extracellular substance; c, cilium; microtubules (arrow). × 10,500. Inset microtubules sectioned transversely and longitudinally. Uranyl acetate × 21,000.

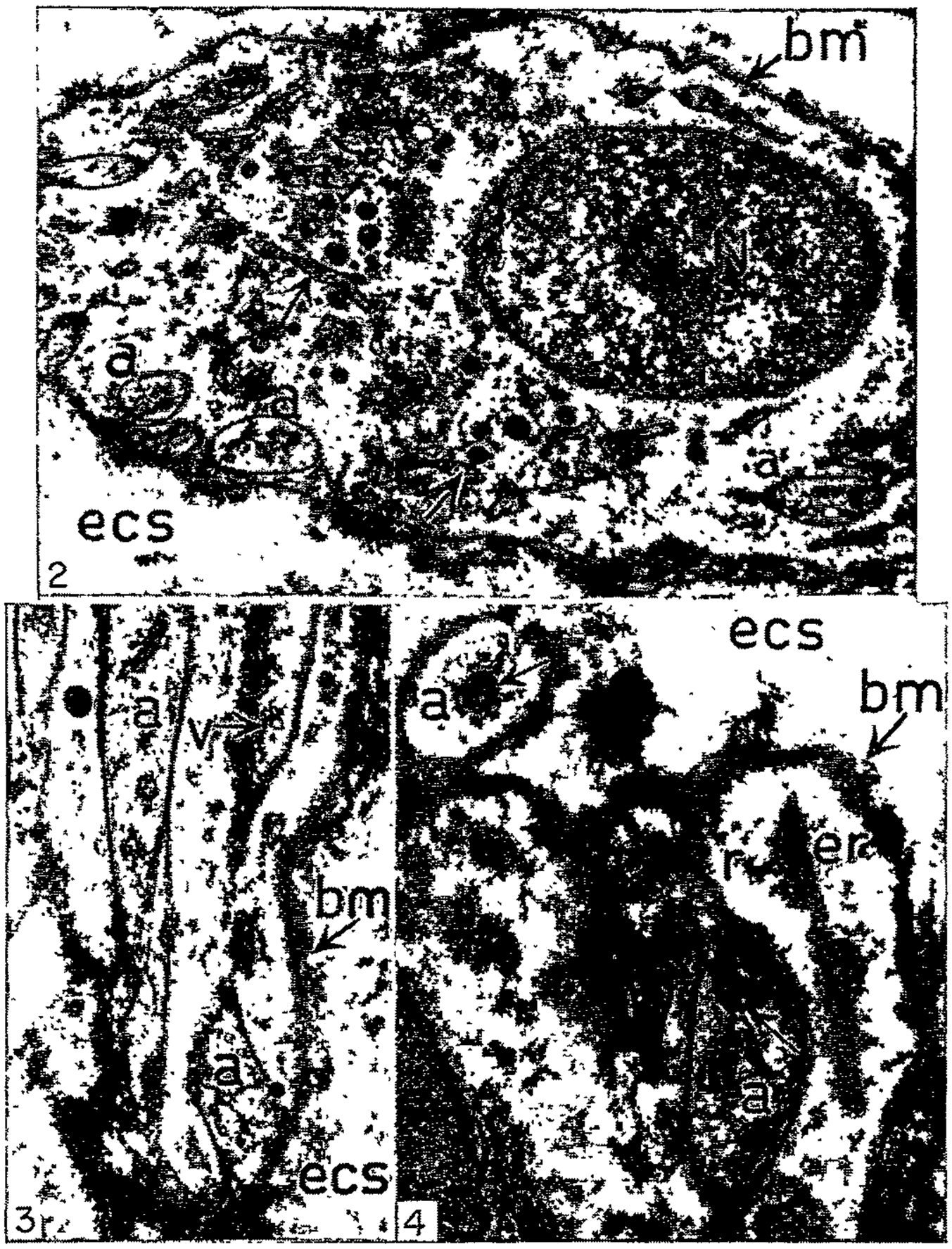
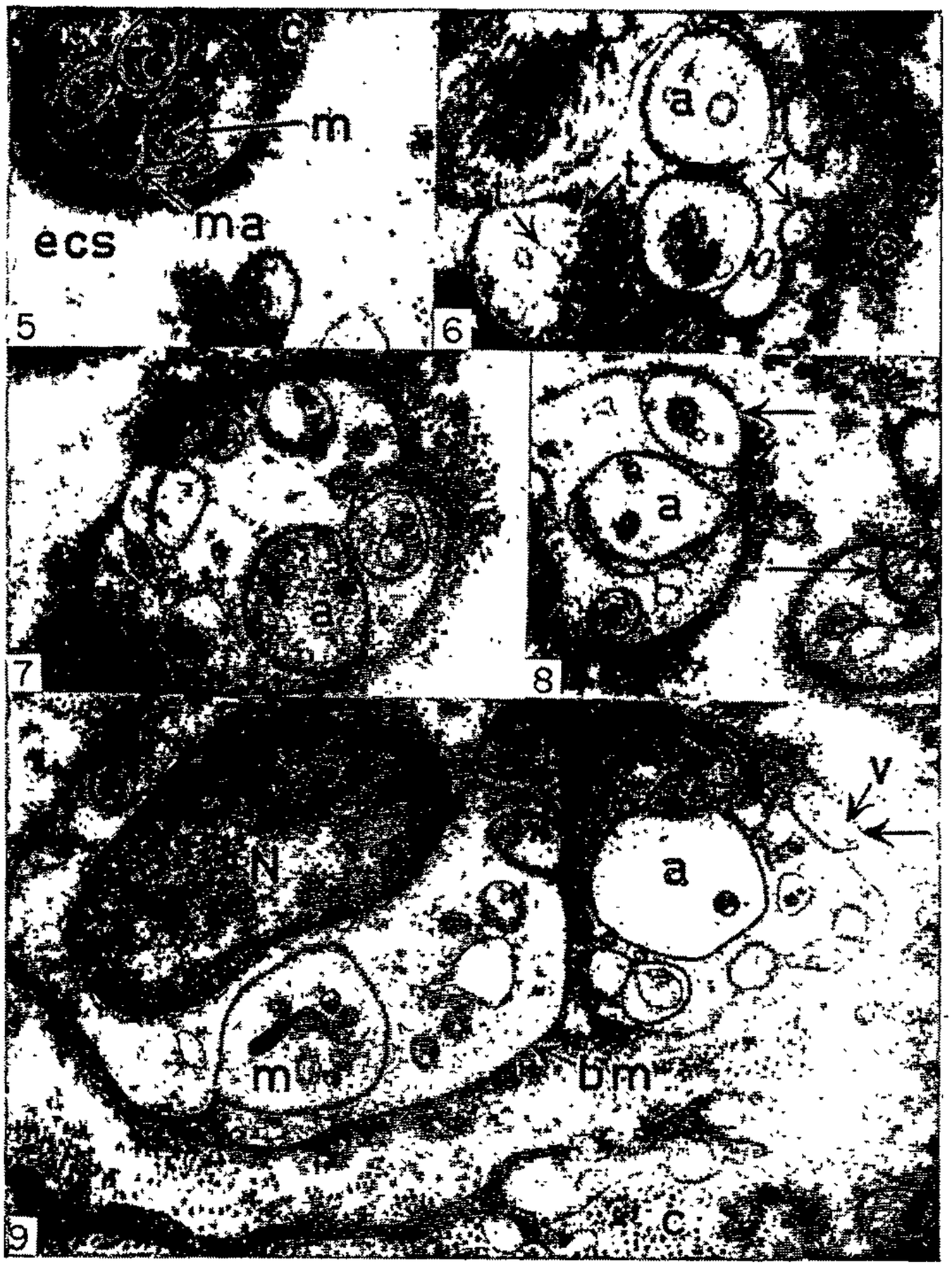


FIG. 2. Schwann cell of developing human periodontium. N, nucleus; a, unmyelinated axons; bm, basement membrane; ecs, extracellular substance; mitochondria (arrows). Uranyl acetate × 4200.

FIGS. 3, 4. Unmyelinated nerve fibres of developing periodontium sectioned longitudinally. a, axons; v, synaptic vesicles; bm, basement membrane; ecs, extracellular substance; er, endoplasmic reticulum; r, ribosomes; mitochondria with electron-dense matrices (arrows). Uranyl acetate × 10,500.

PLATE 2



FIGS. 5–9. Transverse sections of unmyelinated nerve fibres and nerve endings of functional periodontium surrounded by collagen fibrils. ma, mesaxon; m, mitochondria; ecs, extracellular substance; c, collagen fibrils; a, axons; bm, basement membrane; t, microtubules; v, synaptic vesicles; N, nucleus; nerve endings (arrows). Note the variable density of the mitochondrial matrix. Uranyl acetate Figs. 5, 7, 8, 9. × 12,180; Fig. 6, × 22,260.

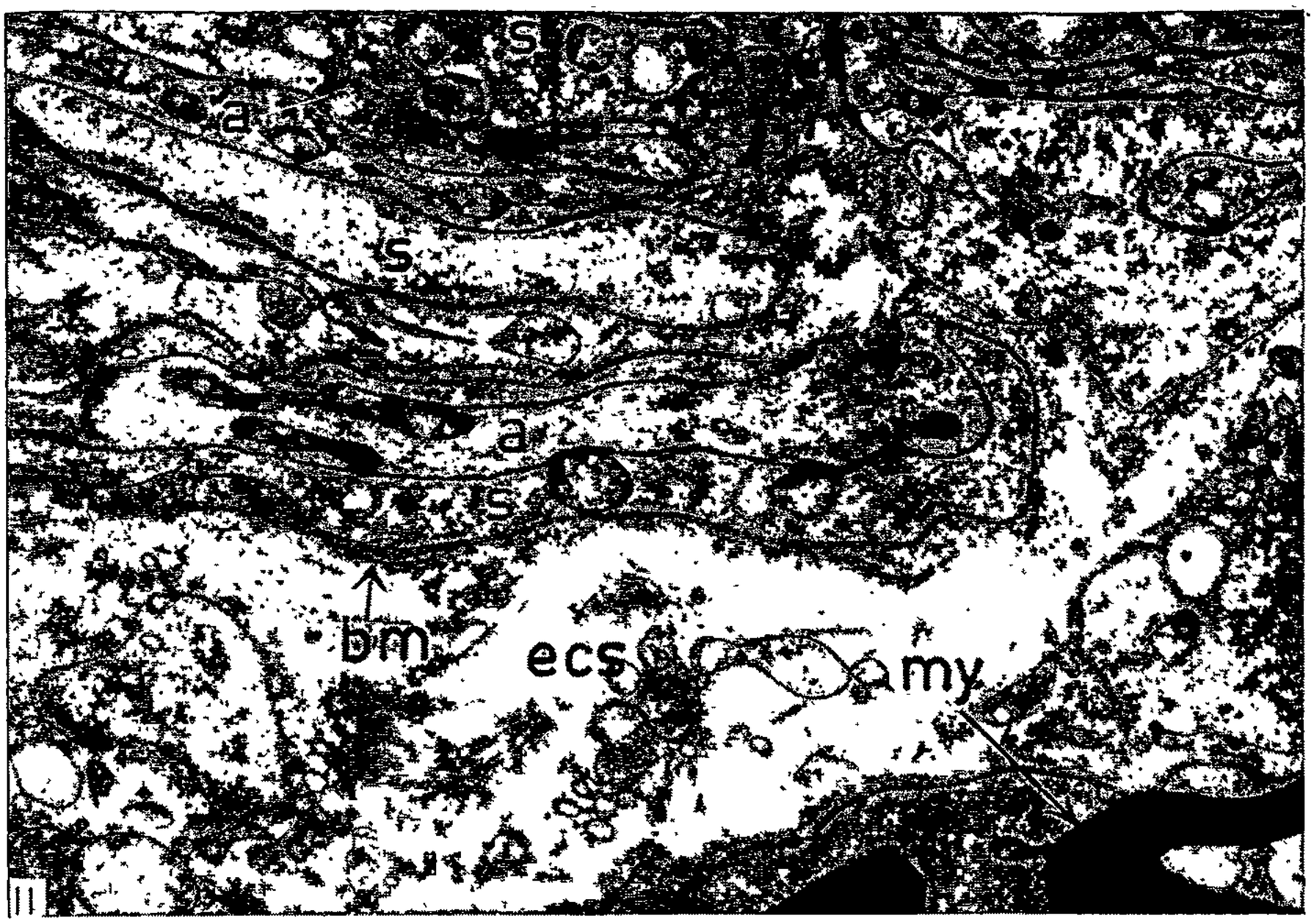
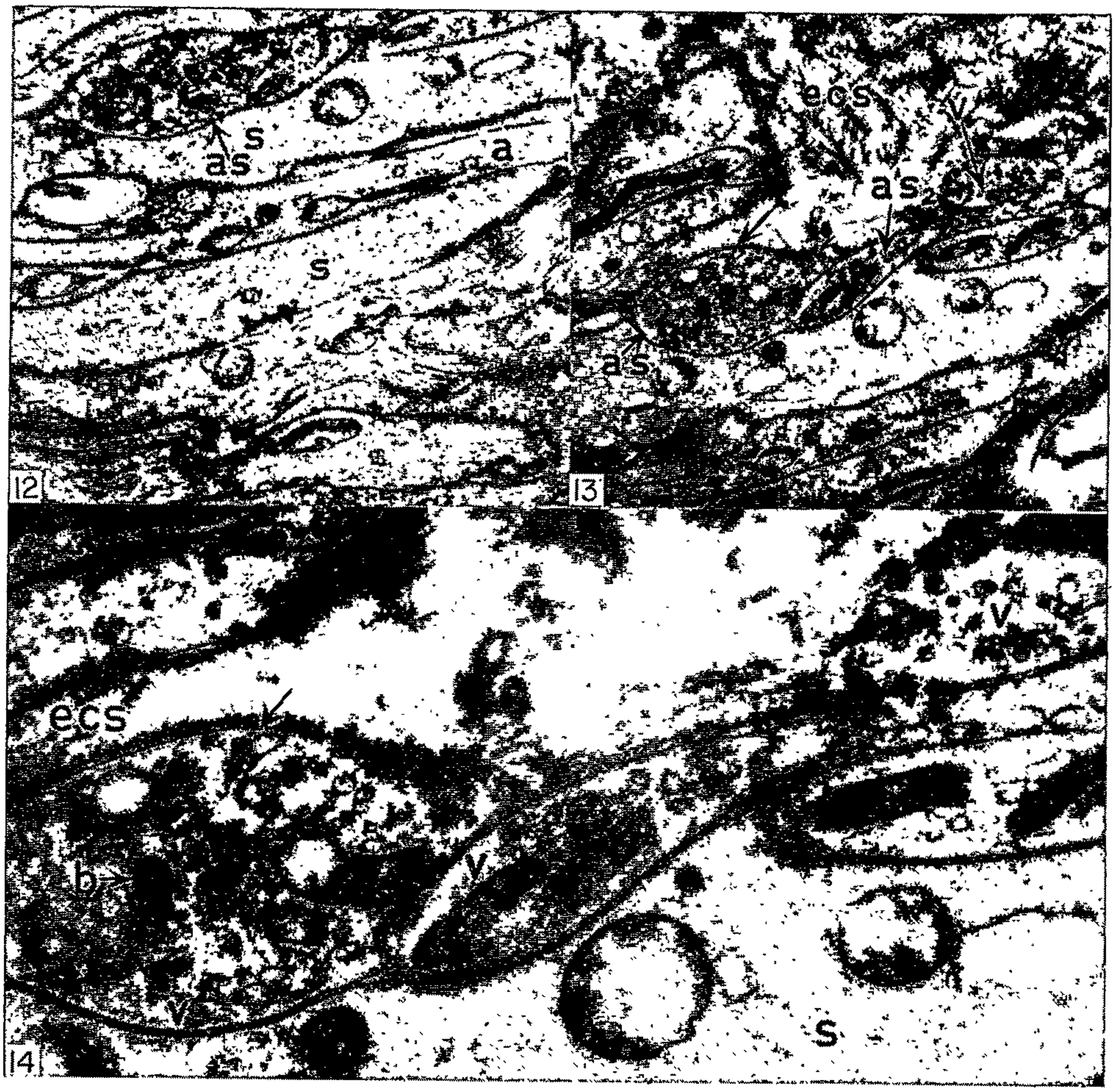


FIG. 11. Longitudinal section of Group III fibres after loss of myelin sheath distal to termination of myelin lamellae. a, axons; s, Schwann cell cytoplasm; my, myelinated nerve fibre; ecs, extracellular substance; bm, basement membrane. Uranyl acetate × 10,920.

PLATE 4



FIGS. 12–13. Representative serial sections of periodontal nerve endings derived from Group III fibres. a, axon of uniform diameter; s, Schwann cell cytoplasm; as, as_1, as_2, axonal terminal swellings exposed in the ground substance; ecs, extracellular substance; v, intra-terminal vesicles; basement membrane of exposed axon (arrow). Uranyl acetate × 10,920.

FIG. 14. Detail of nerve ending in Fig. 13. v, vesicles; b, electron–dense body. Uranyl acetate × 28,350.

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Innervation of human periodontium
I. Classification of periodontal receptors

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ABSTRACT—The neural tissue in human periodontium is associated with the terminal part of a nerve trunk from which myelinated nerve fibres leave and in some instances divide into three or more nerve fibres.

Encapsulated myelinated nerve fibres lose their myelin sheaths and encircle the adjacent myelinated nerve fibres to form compound mechanoreceptors approximately 35×45 μm. Simple mechanoreceptors of approximately 10×10 μm consisted of single myelinated nerve fibres surrounded by cell bodies and terminated as encapsulated unmyelinated nerve fibres. A cluster of compound mechanoreceptors formed a complex approximately 100×150 μm.

An arterial system appeared to supply nutrition for the compound receptors, whilst an arcade of veins surrounded the neural complex.

Introduction

In the period 1935–1937 three papers on the periodontal innervation were published,[1] [2] [3] which formed the basis of all information covering the pressoreceptors in periodontal tissues. At the same time it is noted that the report in 1936 by Brashear[4] formed the basis for the fibre spectrum of the nerve tissue. From that research the pressoreceptors were described either as end rings[1] or knob-like fusiform endings[2] and the fibre spectrum as consisting of nerve fibres of which 20 per cent were over 10 μm in diameter. Subsequently, in 1966 Simpson[5] confirmed the findings of Lewinsky and Stewart and referred to knob-like enlargements and free unmyelinated nerve endings. The investigations cited were confined to light microscopy. The unmyelinated nerve fibre spectrum of periodontal tissue has received scant attention, presumably because of the technical difficulties associated with the estimation of the fibre size.

Wilson,[6] in an electron-microscopic study of the unmyelinated nerve fibre spectrum of the dental branches of the maxillary nerve, found that the fibre size ranged from 0·3 μm to 1·0 μm,

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(1) Berkelbach van der Sprenkel, H.—Microscopical investigation of the innervation of the tooth and its surroundings. Jnl. Anat., 70 : 2, 233–241 (Jan.) 1936.

(2) Lewinsky, W., and Stewart, D.—The innervation of the periodontal membrane. Jnl. Anat., 71 : 1, 98–102 (Oct.) 1937.

(3) Lewinsky, W., and Stewart, D.—The innervation of the periodontal membrane of the cat with some observations on the functions of end-organs found in that structure. Jnl. Anat., 71 : 2, 232–235 (Jan.) 1937.

(4) Brashear, A. D.—The innervation of teeth. Jnl. Comp. Neurol., 64 : 1, 169–185 (June) 1936.

(5) Simpson, H. E.—The innervation of the periodontal membrane as observed by the apoxestic technique. Jnl. Periodont., 37 : 5, 374–376 (Sept.-Oct.) 1966.

(6) Wilson, D.—The maxillary nerve of the cat. Thesis D.D.Sc. degree, University of Sydney, 1968.

whilst Wilson and Silva,[7] in a study of the unmyelinated nerve fibres in the phrenic nerve of the cat, found that external diameters of the fibres also ranged between 0·3 μm and 1 μm. Griffin and Harris,[8] in a study of unmyelinated nerve fibres of the developing and functional human periodontium, found that they had a mean diameter of 0·4 μm and that the largest had diameters of approximately 1 μm.

Physiological experiments by Pfaffmann[9] and Hannam[10] in regard to the conduction velocity of periodontal myelinated nerve fibres have shown that they conduct at 24–83 m/sec. Using the Hursh factor[11] on these velocities, the fibre size would be between 4–14 μm in diameter. However, these authors admitted that the velocity of smaller fibres could be masked by the techniques used.

Histologically, Wilson[6] found that the myelinated nerve fibre spectrum of the maxillary canine nerve of the cat was 3–8 μm, and De Lange, Hannam and Mathews,[12] utilizing physiological techniques, found that the fibre spectrum of the mandibular canine and incisor nerves of the dog was 5–8 μm.

This paper is the first of a series concerned with an analysis of the fibre spectrum, the identification of types of pressoreceptors and free nerve endings in the periodontal tissue. It deals with the structure of periodontal mechanoreceptors.

Materials and methods

Twenty-five freshly extracted human teeth were immediately covered with 1 per cent osmium tetroxide in phosphate buffer pH 7·4. Whilst covered with the fixative, the periodontal ligament was scraped from the root surface and cut into pieces approximately 1 × 1 mm. These pieces of tissue were then immersed in 1 per cent osmium tetroxide in phosphate buffer pH 7·4 for 3 hr at 0° C. They were then dehydrated in increasing concentrations of acetone (25, 50 and 75 per cent) for 15 min each. After rinsing in three changes of acetone, the pieces of tissue were embedded in Araldite. Polymerization was effected at 60° C for 24 hr.

Thick sections were cut from each block and stained with toluidine blue. These sections were examined with the light microscope for encapsulated and free nerve endings. Encapsulated and free nerve endings were found in the periodontal ligament of the lower second molar tooth of a 15-year-old girl and in an upper central incisor of a 12-year-old girl. Each block was then trimmed so that the tissue consisted almost entirely of the encapsulated and free neural complexes.

The sections were cut on an L.K.B. ultramicrotome at 500–1000 Å intervals and stained with uranyl acetate on the grid for 1 hr. Electron micrographs and observations were made on an Hitachi H–S. 7 electron microscope. Wherever possible, serial sections from each block were studied. Observations on these sections will be reported in subsequent papers.

Observations

Periodontal neural tissue was found to be associated with the terminal part of a nerve trunk which consisted of 20–30 nerve fibres surrounded by perineurium, and epineurium (Fig. 1). Myelinated nerve fibres were seen to leave the nerve trunk and on occasions to divide into three or more terminal nerve fibres (Fig. 2). When division of the parent fibre occurred, the terminal branches were immediately encapsulated (Fig. 2). The encapsulated myelinated nerve fibres when traced distally (Fig. 2, 4a, b) were seen to lose their myelin sheaths in a staggered fashion and surround or encircle the adjacent myelinated nerve fibres of the complex (Fig. 4a, b). We have called these endings endings compound mechanoreceptors or end rings. The dimensions of these receptors were in the range 35 × 45 μm. Associated with or as isolated structures were simple mechanoreceptors which consist of a single myelinated nerve fibre surrounded by cell bodies and processes of capsular cells which when followed distally was seen to lose its myelin sheath and to terminate as encapsulated unmyelinated nerve fibres (Fig. 3, 4). The dimensions of the simple receptors were in the range 10 × 10 μm. The compound mechanoreceptor although surrounded by cell bodies and processes of capsular cells was in intimate association with the surrounding dense periodontal tissue, whilst the simple mechano-

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[7] Wilson, A. S., and Silva, D. G.—Ultrastructure of the phrenic nerve. Nature, 208: 5011, 707–708 (Nov. 13) 1965.

[8] Griffin, C. J., and Harris, R.—Unmyelinated nerve endings in the periodontal membrane of human teeth. Arch. Oral Biol., 13: 10, 1207–1212 (Oct.) 1968.

[9] Pfaffmann, C.—Afferent impulses from the teeth due to pressure and noxious stimuli. J. Physiol. (London), 97: 2, 207–219 (Dec. 14) 1939.

[10] Hannam, A. G.—The conduction velocity of nerve impulses from dental mechanoreceptors in the dog. Arch. Oral Biol., 13: 11, 1377–1383 (Nov.) 1968.

[11] Hursh, J. B.—Conduction velocity and diameter of nerve fibres. Amer. J. Physiol., 127: 1, 131–139 (Aug.) 1939.

[12] De Lange, A. A., Hannam, A. G., and Mathews, B.—The diameters and conduction velocities of fibres in the terminal branches of the inferior dental nerve. Arch. Oral Biol., 14: 5, 513–519 (May) 1969.

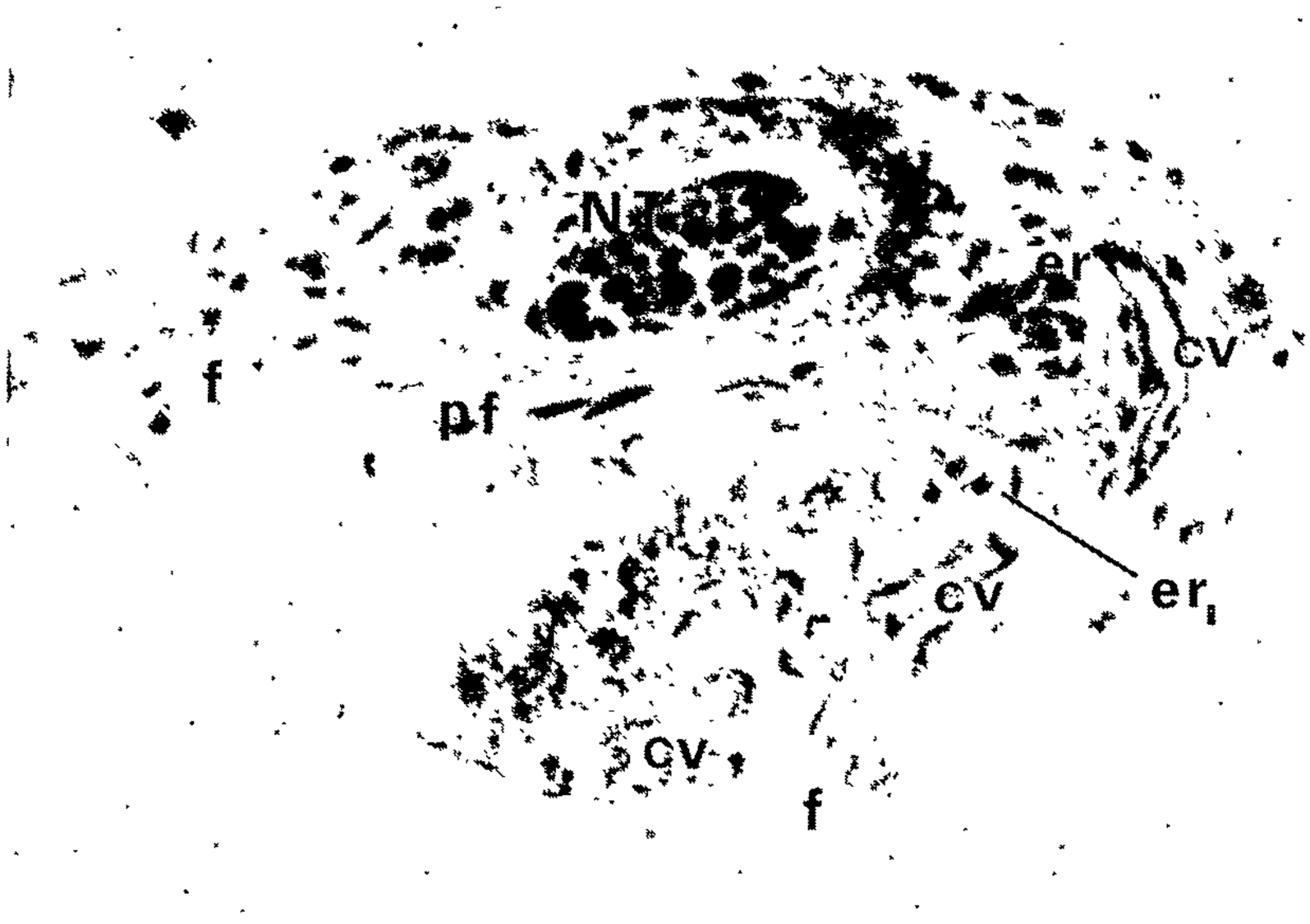

Fig. 1.—Periodontal neural tissue. NT, periodontal nerve trunk; cv, collecting veins; er, proximal part of end ring; pf, parent nerve fibre showing terminal branching; f, dense fibrous tissue. ×500.

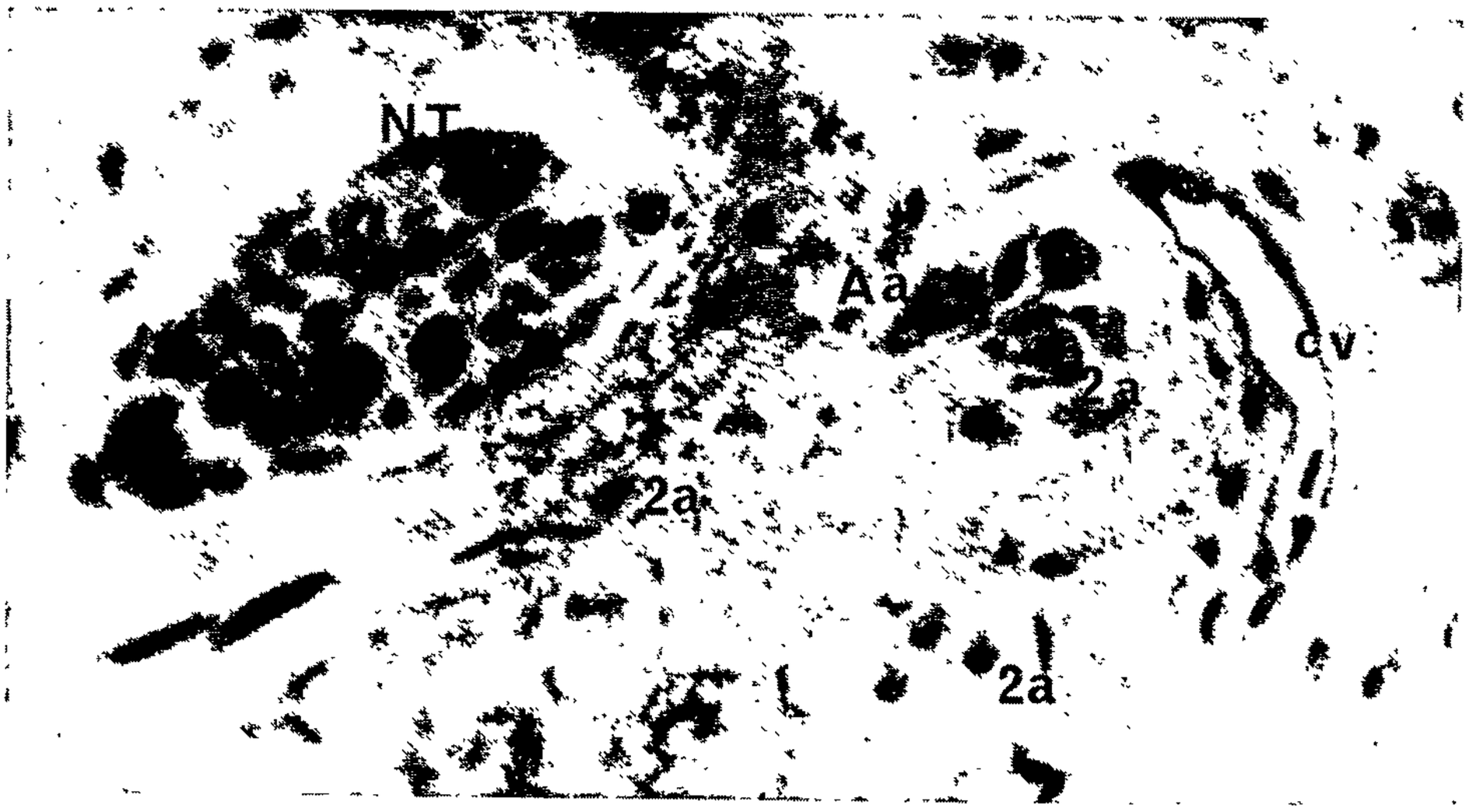

Fig. 2.—Detail from Fig. 1. NT, periodontal nerve trunk; Aa, afferent arteriole; cv, collecting vein; 2a, proximal and distal portions of periodontal end ring. ×1,200

receptors were surrounded by very loose connective tissue (Fig. 3, 4).

Capsular cell bodies and processes were associated with the entire neural complex and formed a reticulum encompassing both the simple and compound mechanoreceptors. In one instance a capsular cell body was seen interposed between two simple mechanoreceptors (Fig. 4).

Surrounding the neural complex was an arcade of veins (Fig. 1, 2) which have been termed collecting

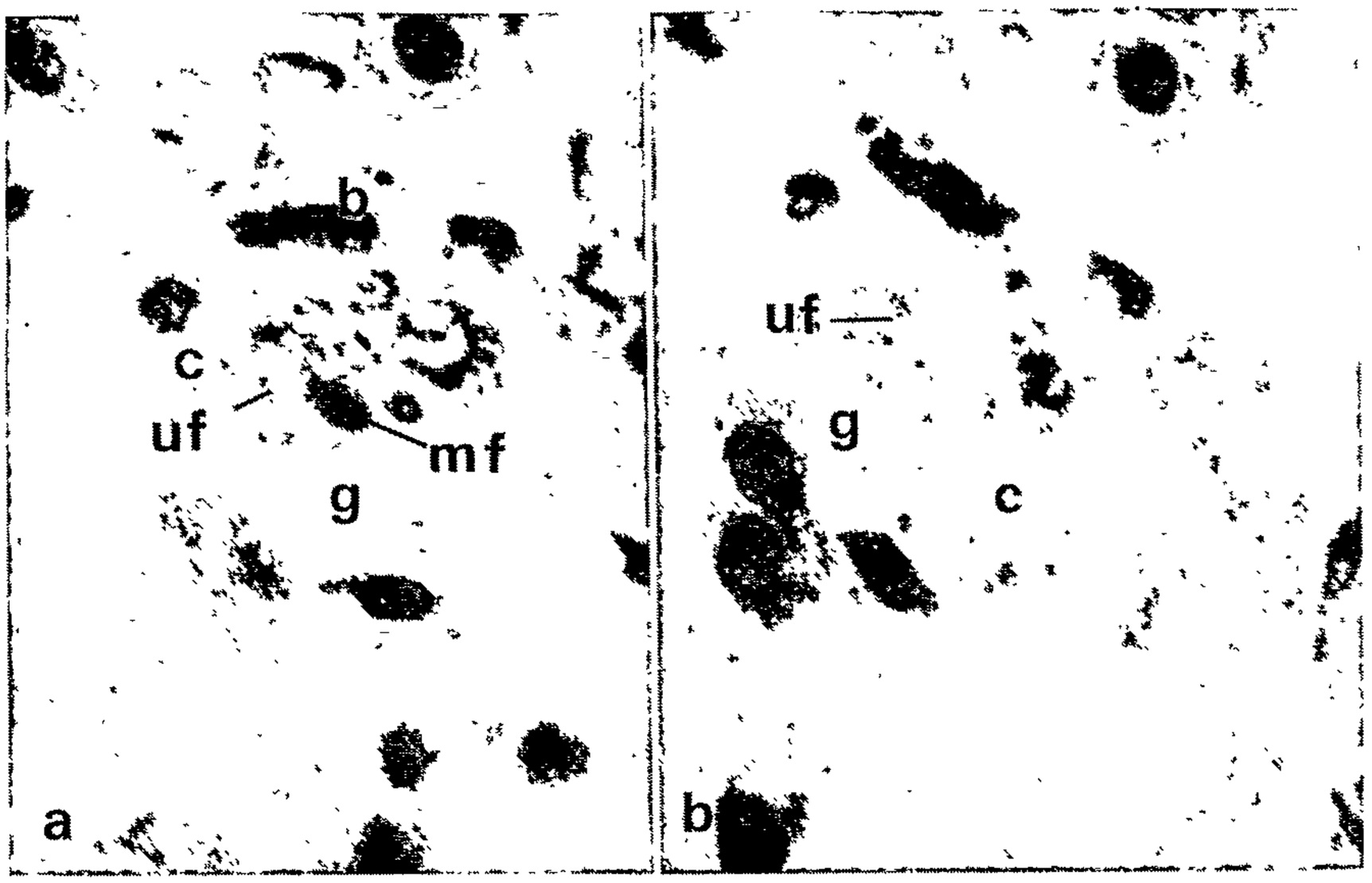

Fig. 3.—Detail of simple mechanoreceptor. (a) Proximal part. mf, myelinated nerve fibre; uf, unmyelinated nerve fibre; c, capsule of simple mechanoreceptor; g, ground substance; b, cell bodies of capsular cells. ×1,800. (b) Distal part (4 μm distal to (a)). uf, unmyelinated nerve fibres; c, capsule; g, ground substance. ×1,800.

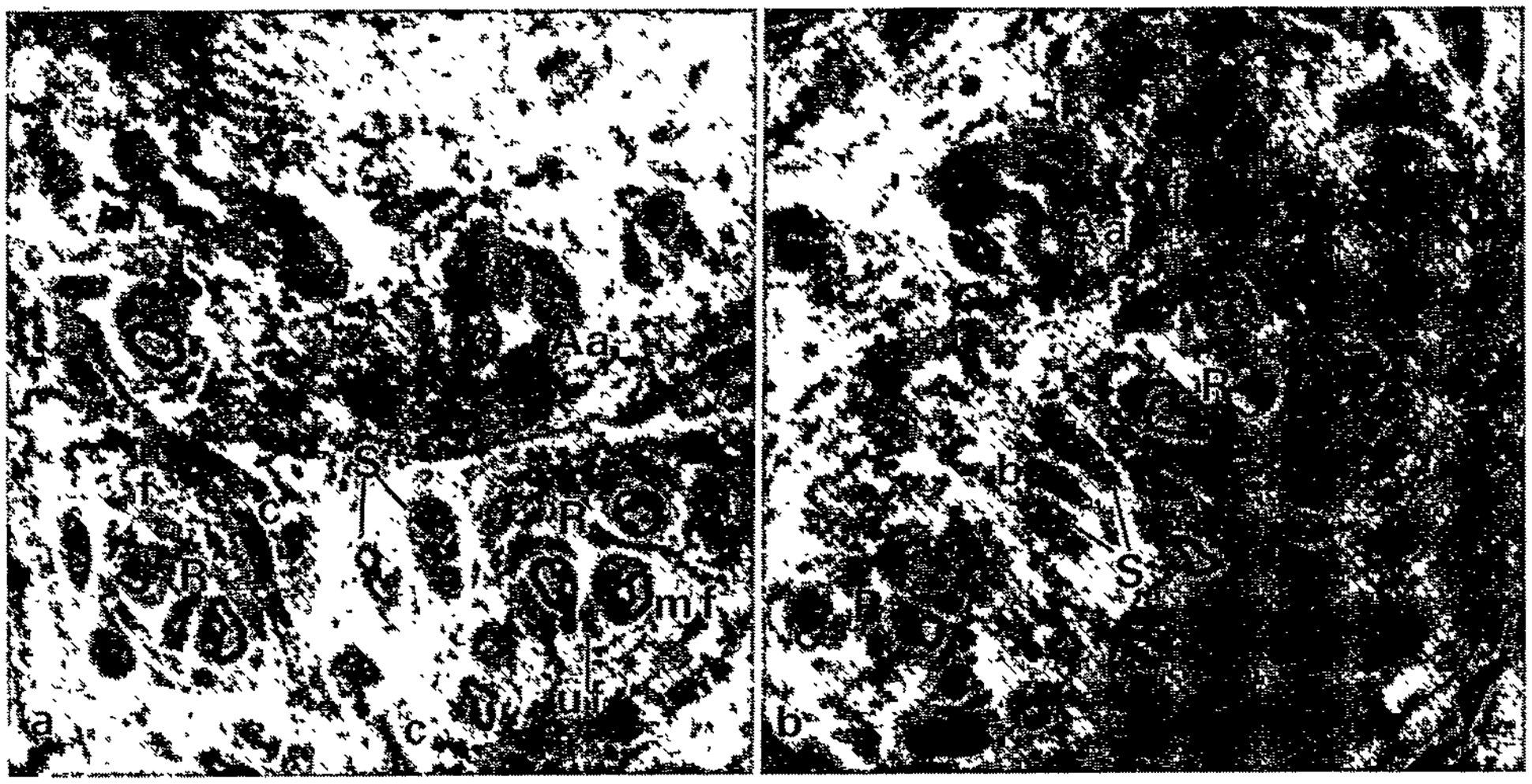

Fig. 4.—Simple and compound mechanoreceptors. (a) Proximal region to neural complex. R, compound receptor; mf, myelinated nerve fibres of compound receptor; uf, unmyelinated nerve fibres of compound receptor forming rings around myelinated nerve fibres; c, cell body of capsular cell surrounding compound receptors; S, proximal and distal parts of two simple receptors; Aa, afferent arteriole. ×1,800. (b) Distal region of neural complex (4 μm distal to (a)). R, compound receptors; S, simple receptors; Aa, afferent arteriole; b, cell body of capsular cell interposed between two simple mechanoreceptors. ×1,800.

veins, and blood vessels of the arteriolar and metarteriolar type were seen to be in close proximity to the compound mechanoreceptor (Fig. 2, 4).

The periodontal nerve trunk when followed distally showed a marked decrease in the number of myelinated nerve fibres. In part, this seemed to occur because some of the fibres left the main trunk to terminate as simple or compound mechanoreceptors and in part because some of the fibres had lost their myelin sheaths in a staggered fashion and terminated as a cluster of compound mechanoreceptors (Fig. 5). We have termed this receptor the complex mechanoreceptor, and its dimensions are approximately 100 × 150 μm.

Discussion

1. *Simple mechanoreceptors*

Simple mechanoreceptors were seen as isolated receptors derived from a single myelinated fibre and surrounded by loose connective tissue (Fig. 3, 4).

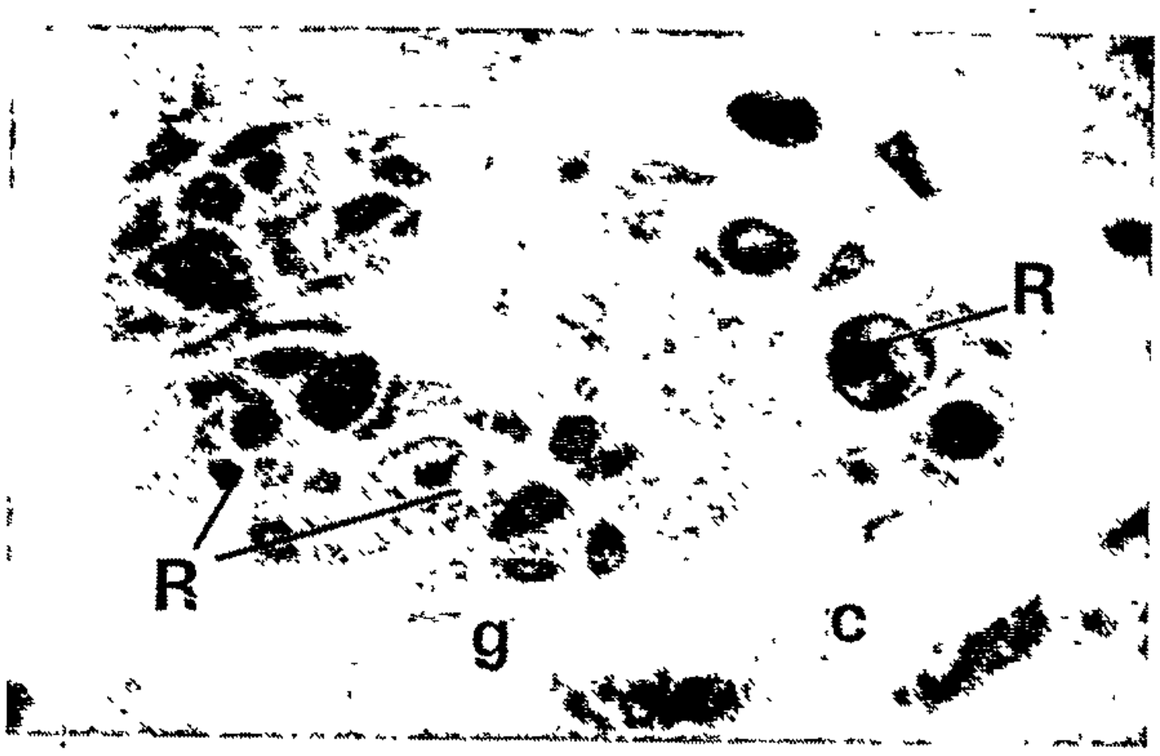

Fig. 5.—Distal portion of periodontal nerve trunk terminating in a cluster of mechanoreceptors (complex). R, compound receptors; c, capsule; g, ground substance. ×1,800. cf with proximal part seen in Fig. 2.

The amount of loose connective tissue surrounding the receptor was approximately equal to the size of the receptor, which suggests that the latter could move away from an applied force and thus corresponds to a rapidly adapting unit. Thus it would seem to correspond to units mediating the jaw opening reflex; usually after tooth contact there is reflex inhibition of the mandibular elevators for approximately 10 m/sec.[13]

Jerge[14] was able to obtain action potentials from neurones in the mesencephalic nucleus of the trigeminal nerve when pressure was applied to a single tooth or a group of teeth. He identified the receptors as Type I and Type II dental pressoreceptors respectively. The latency of the response suggested that Group II nerve fibres were involved. Most authors agree[9] [14] [15] [16] [17] [18] that there is a preferred direction for the application of force to the tooth which produces the greatest response. Kawamura and Nishiyama[16] made the observation that although there was an optimal direction which stimulated the tooth neurones with the lowest threshold, nevertheless most tooth neurones were sensitive to pressure from many directions.

2. *Compound and complex mechanoreceptors*

The compound and complex mechanoreceptors have the same structure but differ in the larger dimensions of the complex mechanoreceptors. The architecture of these receptors suggests that the function is to sample tension in the periodontal membrane. Their essential feature is the encirclement of adjacent myelinated fibres by the unmyelinated nerve fibres of the receptor (Fig. 4, 5). This would indicate that a stretch of any one nerve fibre of the complex would excite the other elements of the receptor; because of this these receptors could be responsible for the spontaneous discharges elicited from the periodontium of the dog.[19]

3. *Neural complex*

The periodontal neural complex has been represented schematically in Fig. 6. The main nerve trunk consists of myelinated and unmyelinated nerve fibres. The unmyelinated nerve fibres leave the main trunk and terminate as free endings in the connective tissue[20] or as vasomotor and DRC (dorsal root C) fibres (Group IV). Myelinated nerve fibres leave the main nerve trunk and terminate freely in the tissues (Group III).[8]

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[13] Griffin, C. J., and Munro, R. R.—Electromyography of the jaw-closing muscles in the open-close-clench cycle in man. Arch. Oral Biol., 14: 2, 141–150 (Feb.) 1969.

[14] Jerge, C. R.—Organization and function of the trigeminal mesencephalic nucleus. J. Neuro. Physiol., 26: 3, 379–392 (May) 1963.

[15] Kruger, L., and Michel, F.—A single neurone analysis of the buccal cavity representation in the sensory trigeminal complex. Arch. Oral Biol., 7: 4, 491–503 (July-Aug.) 1962.

[16] Kawamura, Y., and Nishiyama, T.—Projection of dental afferents to the trigeminal nuclei of the cat. Jap. J. Physiol., 16: 584–597, 1966.

[17] Ness, A. R.—The mechanoreceptors of the rabbit mandibular incisors. J. Physiol. Lond., 126: 3, 475–493 (Dec.) 1954.

[18] Hannam, A. G.—The response of periodontal mechanoreceptors in the dog to controlled loading of the teeth. Arch. Oral Biol., 14: 7, 781–791 (July) 1969.

[19] Hannam, A. G.—Spontaneous activity in dental mechanosensitive units in the dog. Arch. Oral Biol., 14: 7, 793–801 (July) 1969.

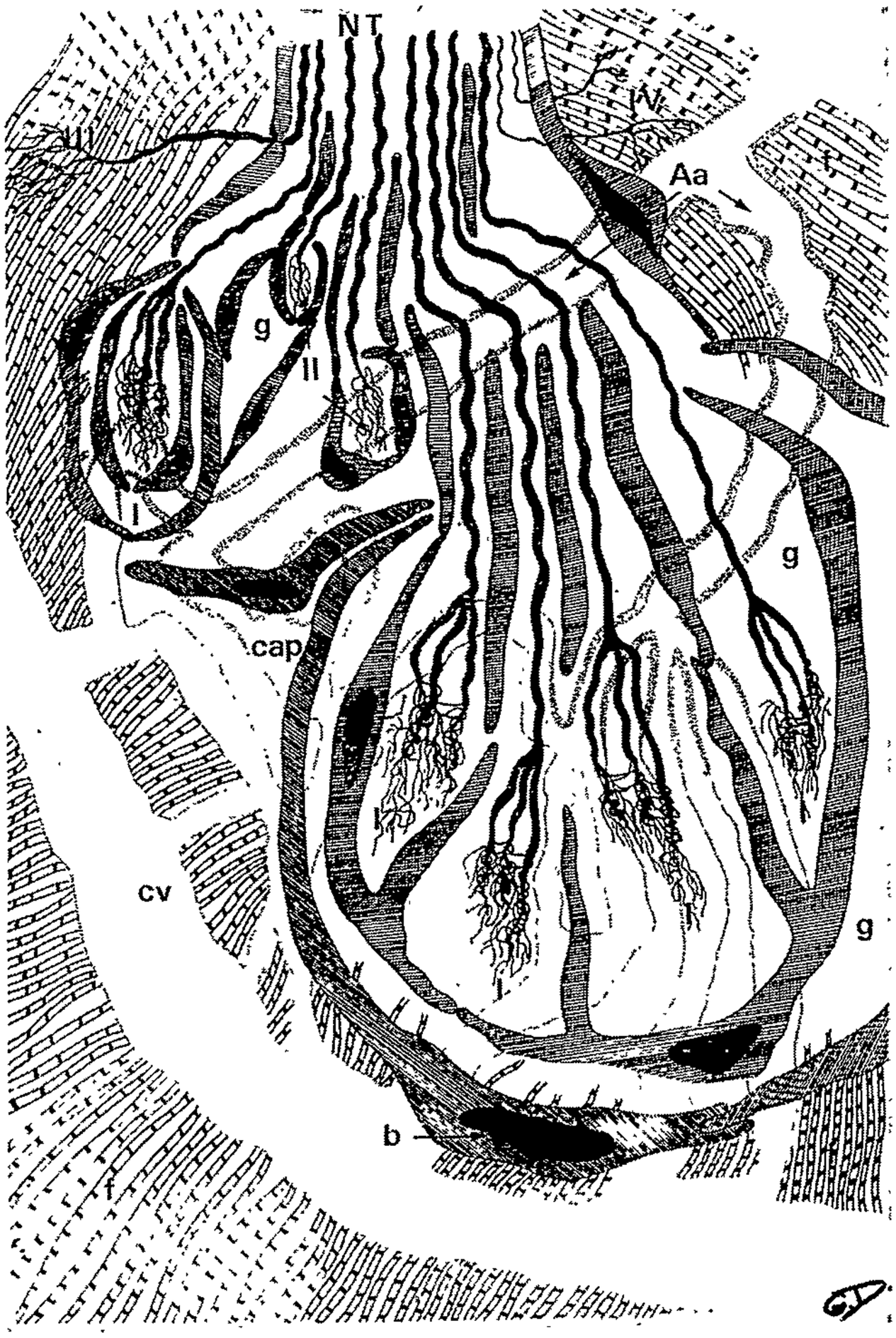

Fig. 6.—Schematic representation of periodontal neural tissue. I, compound mechanoreceptors derived from either Group II or III nerve fibres occurring singly or forming a cluster (complex); II, simple mechanoreceptor derived from Group II and III nerve fibres; III, ending of Group III nerve fibres; IV, ending of Group IV nerve (vasomotor and DRC) fibres; NT, distal part of periodontal nerve trunk; Aa, afferent arteriole; cv, collecting vein; cap, capillary system; b, cell bodies and processes of capsular cells forming a reticulum; g, ground substance; f, dense fibrous tissue.

The encapsulated receptors are derived from myelinated nerve fibres and terminate as either simple or compound mechanoreceptors. The complex ending is shown as a cluster of compound endings.

The simple mechanoreceptor would seem to correspond to the end knobs of Lewinsky and Stewart[3] and be responsible for the jaw opening reflex, whereas the compound and complex endings would seem to correspond to the end rings described by Berkelbach van der Sprenkel.[1] The vascularization of the complex is represented by an afferent arteriole directly nourishing the compound and complex receptors, but the simple receptors receive their nutrition by diffusion.

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