Ultrastructural changes in pressure zones of human periodontium incident to orthodontic tooth movement

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In order to characterize the ultrastructural alterations of cells, vasculature and fibrils that occur on the pressure side of the periodontal ligament when teeth are subjected to orthodontic forces of a magnitude generally used in clinical practice, eleven premolar teeth were moved buccally by means of a fixed appliance. The experimental periods were 2, 21 and 50 days. Previous light microscopic observations of cellular and vascular disturbances in compressed zones of the periodontal ligament were verified. In addition, ultrastructural details of the process of degeneration and necrosis of cells and vascular elements were obtained. It was demonstrated that cells and blood vessels in the hyalinized zones are damaged to an extent where restitution to normal function is inconceivable. Although collagen fibrils disintegrated by longitudinal splitting into filaments without periodicity, the majority of fibrils retained the cross striations. Apart from differences related to the time factor, there was close agreement between the results of the present study on human material and in recent studies on rats where structural alterations have been systematically observed.

Key-words: Microscopy, electron; orthodontics; periodontium

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Development of cell-free, structureless, so-called hyalinized zones in the periodontal ligament on the pressure side of teeth subjected to orthodontic forces has been reported by many authors (*Sandstedt*, 1904; 1905; *Schwarz*, 1932; *Skillen & Reitan*, 1940; *Oppenheim*, 1944; *Macapanpan, Weinmann & Brodie*, 1954). Apparently, a certain degree of hyalinization must be expected in all clinical orthodontic work, depending on anatomical characteristics and the magnitude of the force (*Reitan*, 1969).

The observation that extensive hyalinization leads to a standstill of tooth movement (*Skillen & Reitan*, 1940) and indications that root resorptions may occur in connection with compressed areas of the periodontal ligament (*Kvam*, 1972; *Reitan*, 1972) indicate that a further analysis of the development and fine structure of these areas might improve the understanding of tissue reactions incident to tooth movement.

Some investigators have maintained that the osteocytes of the alveolar bone adjacent to hyalinized zones degenerate, leaving empty lacunae (*Oppenheim*, 1944; *Macapanpan et al.*, 1954), and these bone areas have been described as necrotic (*Picton*, 1969). Further studies of the alveolar bone adjacent to hyalinized perio-

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dontal ligament are necessary to clarify the mechanisms involved.

The contention that occurrence of hyalinized zones are of importance in the pathogenesis of periodontal disease (*Glickman*, 1972) indicates that such studies are of interest also from a periodontal point of view.

Recently, transmission electron microscopy has been applied to the study of compression of the periodontal ligament following orthodontic tooth movement. Ultrastructural vascular (*Rygh*, 1972a; *Rygh & Selvig*, 1973) and cellular (*Rygh*, 1972b) reactions have been reported in experimental studies on tooth movement in rats.

The purpose of the present investigation was to observe, at the ultrastructural level, the changes of the periodontal ligament and adjacent alveolar bone which occur in orthodontic pressure zones in humans at selected experimental periods, following the application of force of a magnitude generally used in clinical practice.

MATERIAL AND METHODS

Eleven premolar teeth were moved buccally by means of a fixed appliance. A control group of 2 premolar teeth was included. All patients were 13 years of age. Each tooth and supporting tissue was removed after 2, 21 and 50 days.

A force of 70 g was applied in the 2- and 21-days experiments. Two heavier experimental forces were included in the 21-days group; 120 and 240 g respectively; while 100 g was used in the experiments of 50 days duration. The experimental appliance consisted of a spring fixed buccally to the first molar band and ligated to the experimental premolar. The thickness of the spring varied between .014" and .018". The force exerted was measured with a Correx® * instrument and was controlled twice a week. This type of force will produce a compressed zone in the marginal area on the buccal side. At the end of the experimental period the teeth were removed with a small buccal bone plate, the outline of which corresponded to the subjacent pressure zone, adhering to the root surface.

The specimens were processed for electron microscopic examination by techniques previously described (*Rygh*, 1972a, 1972b). Thin sections of the periodontal ligament were made in a buccolingual plane parallel to the long axis of the teeth.

RESULTS

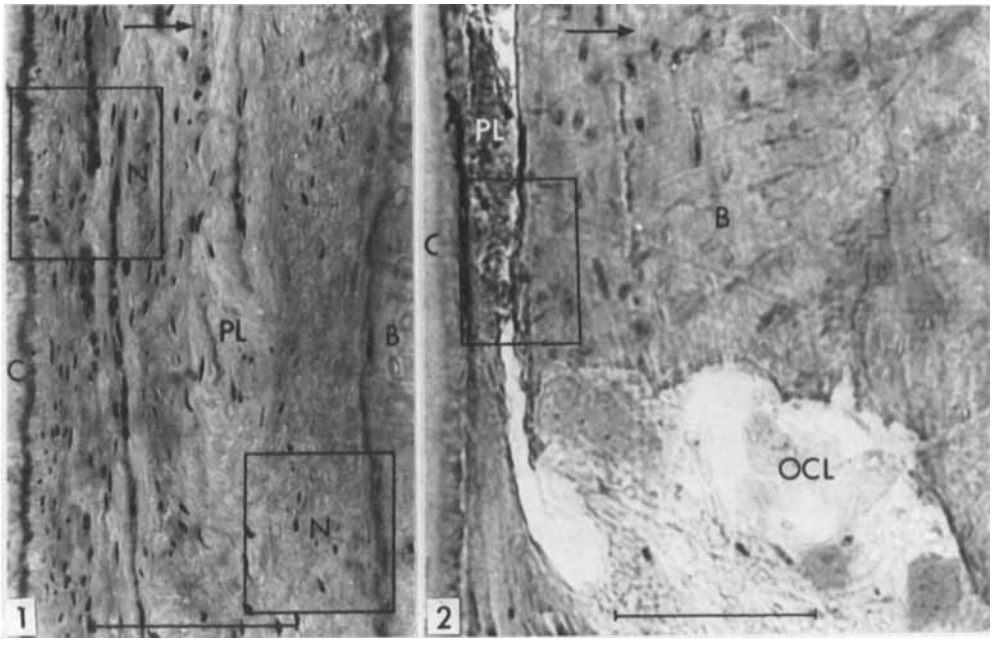
LIGHT MICROSCOPY

Periodontal ligament

2 days. Application of a 70 g force led to compression of the periodontal ligament near the alveolar margin, with a reduction of the width to 150—200 μm. Sections stained with toluidine blue revealed light, homogeneous areas near the alveolar bone (Fig. 1). In these areas most of the cells seemed to have disappeared. Along the root surface, shrunken and densely stained cellular structures could be observed, indicating pyknosis of the cell nuclei. Compressed blood vessels appeared as dark strands lined with heavily stained cellular elements.

21 days. After application of a 70 g force for three weeks, the width of the periodontal ligament in the compressed marginal region had been reduced to 20—40 μm. Between the tooth and the alveolar bone, unidentifiable structures appeared, interspersed with some heavily

* Correx®, Haag-Streit A.G. Bern.



Figs. 1—2. Pressure zones in the marginal area of the periodontal ligament, PL, on the buccal side of the first premolar. Force 70 g. Razor blade sections, stained with toluidine blue. B, alveolar bone. C, cementum. Arrow, direction of force. Areas selected for electron microscopy are marked with boxes. Fig. 1. Light homogeneous area near the alveolar bone. Near the cementum, pyknosis of fibroblasts and endothelial cell nuclei, N. Duration 2 days × 250, bar = 100 μm. Details from this specimen are illustrated in figs. 4—11. Fig. 2. Narrow compressed zone with unidentifiable structures of periodontal ligament bordered by a zone of repair with osteoclastic breakdown, OCL, of alveolar bone. Duration 21 days × 250, bar = 100 μm. Details from this specimen are illustrated in fig. 14.

stained spots which apparently represented cell nuclei (Fig. 2).

The narrow, compressed areas of the periodontal ligament were bordered by a region of osteoclastic breakdown of the alveolar bone. In these areas the width of the periodontal ligament ranged between 250 and 350 μm. Application of a 120 g force led to a more extensive area of hyalinization, but the width of the periodontal ligament was not appreciably reduced as compared to that obtained with a 70 g force.

50 days. While the periodontal ligament in the area of compression was in phase of repair in two specimens, a small hyalinized zone, measuring 150 μm in vertical direction remained in one case (Fig. 15). A layer, consisting of compressed fibrous tissue with a thickness of 5—10 μm, remained adjacent to the cementum surface. The fibrils of the compressed periodontal ligament appeared as strands oriented parallel to the root surface and were heavily stained, while the non-fibrous tissue elements seemed to have disappeared.

Alveolar bone

The osteocytes of the alveolar bone immediately adjacent to the narrow compressed areas of the periodontal ligament appeared as dark spots, frequently surrounded by a clear zone, within lacunae that were distinctly delineated by a heavily stained lacunae wall (Fig. 2).

ELECTRON MICROSCOPY

Periodontal ligament cells

2 days. The ultrastructural appearance of the pressure zones varied. In areas of relatively loose texture which were dominated by cellular structures, i.e. in the vicinity of vascular elements, the extracellular milieu was characterized by oedema, as indicated by an increased manifestation of clear spaces devoid of formed elements (Fig. 4). While the osteoblasts, fibroblasts and cementoblasts from untreated material contained a well developed rough-surfaced endoplasmic reticulum which appeared as flattened or moderately dilated cisternae (Fig. 3), cellular swelling with marked dilation of the endoplasmic reticulum was more prominent in the compressed zones. In these cells vacuoles of varying size containing some stained material, and densely stained globuli, occurred in the cytoplasm (Fig. 5). In cells exhibiting extensive swelling, the limiting membrane was often obscure.

In areas where cells were interspersed between densely packed fibrils, such as near the cementum; naked nuclei, or nuclei with scant remnants of cytoplasmic components remained (Fig. 6); whereas in looser areas dissolution or disintegration of the cytoplasmic membrane had led to a partial or complete loss of cytoplasm (Fig. 7).

The isolated nuclei revealed considerable variation of contour and state of content. Some nuclei showed signs of shrinkage with an irregular outline and aggregation of chromatin along the inner aspect of the nuclear membrane (Fig. 7). The interchromatic spaces were empty and the perinuclear envelope showed points of rupture. Other nuclei demonstrated only a few indentations of the nuclear membrane and contained a loosely packed flocculent material of low density (Fig. 8).

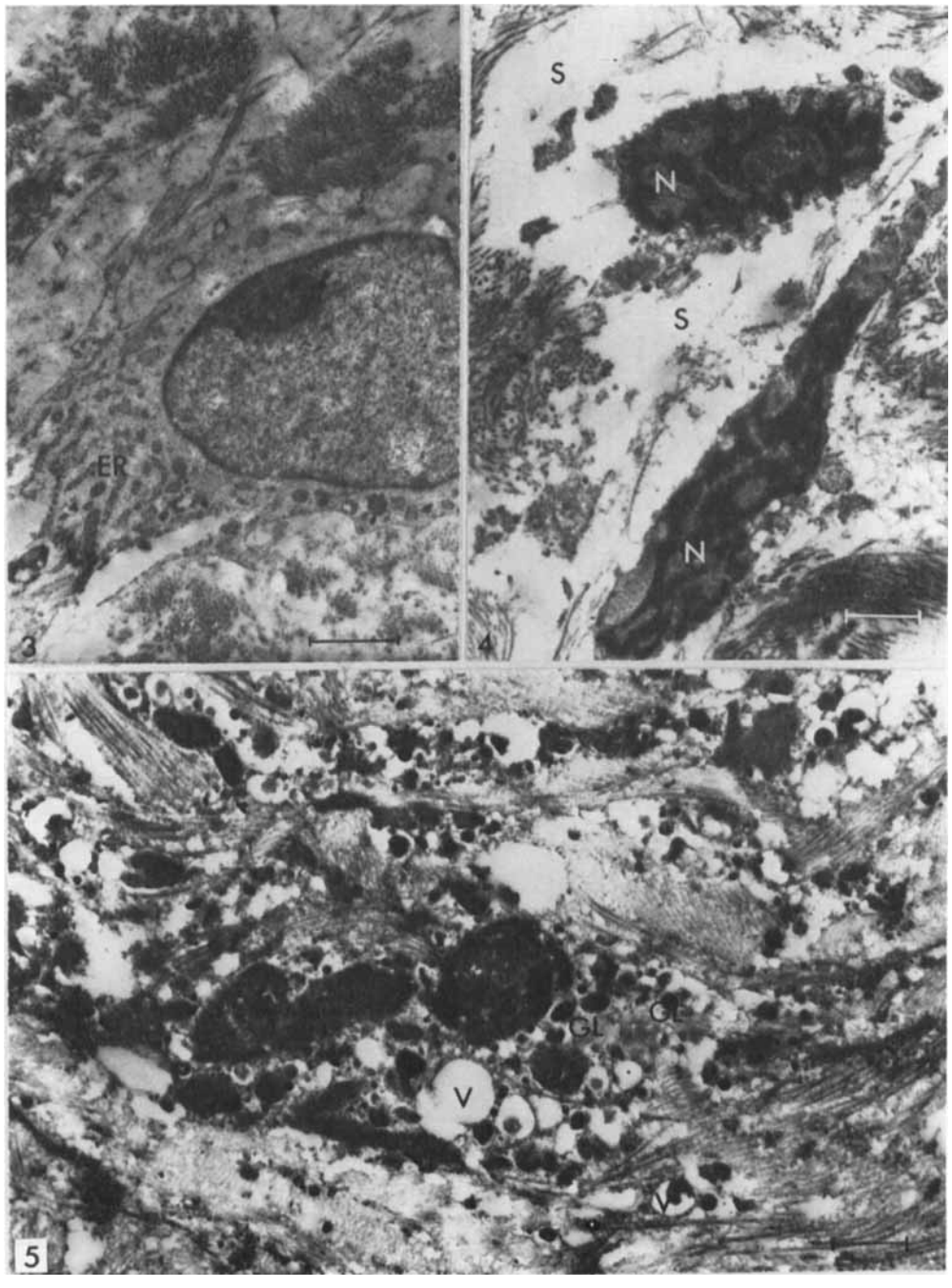
21 and 50 days. Remnants of isolated nuclei were observed in densely compressed areas of the periodontal ligament. Although nuclear fragments were not frequently observed, they did occur in areas which appeared to be completely cell-free when viewed in the light microscope.

Periodontal ligament vascularity

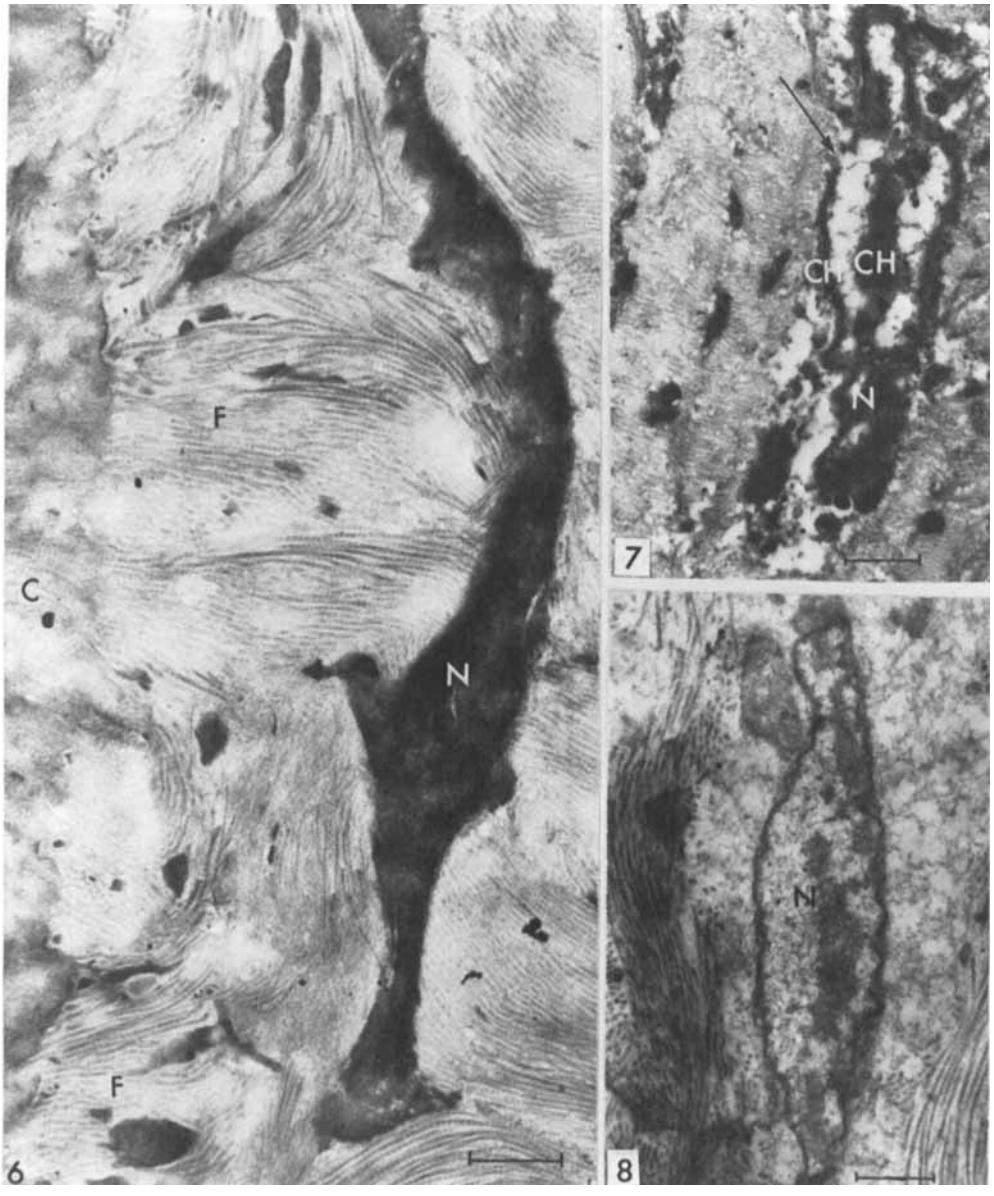
2 days. Slowing of blood circulation or total hemostasis was observed in the compressed zones. Masses of irregularly shaped erythrocytes, exhibiting varying electron density, filled the lumen of the dilated blood vessels. The red blood cells were bordered by round structures of the same electron density (Fig. 9). The endothelial cells exhibited varying stages of disintegration, characterized by intracellular swelling. Advanced dilation of the membraneous systems, separation of the nucleus from the cytoplasm by a bright zone, and formation of clear vacuoles reflected injury to the cells (Fig. 10). Densely stained globuli filled the cytoplasm. The cell organelles frequently retained their distended limiting membranes. Extensive spaces devoid of identifiable contents were observed between cells.

Disintegration of vessel walls was also frequently observed. Parts of the endothelial walls had disappeared, facilitating communication between the lumen and the perivascular structures (Fig. 11).

21 and 50 days. Although remnants of cellular structures could be observed, no definite identification of vascular elements was possible at this late stage of hyalinization.



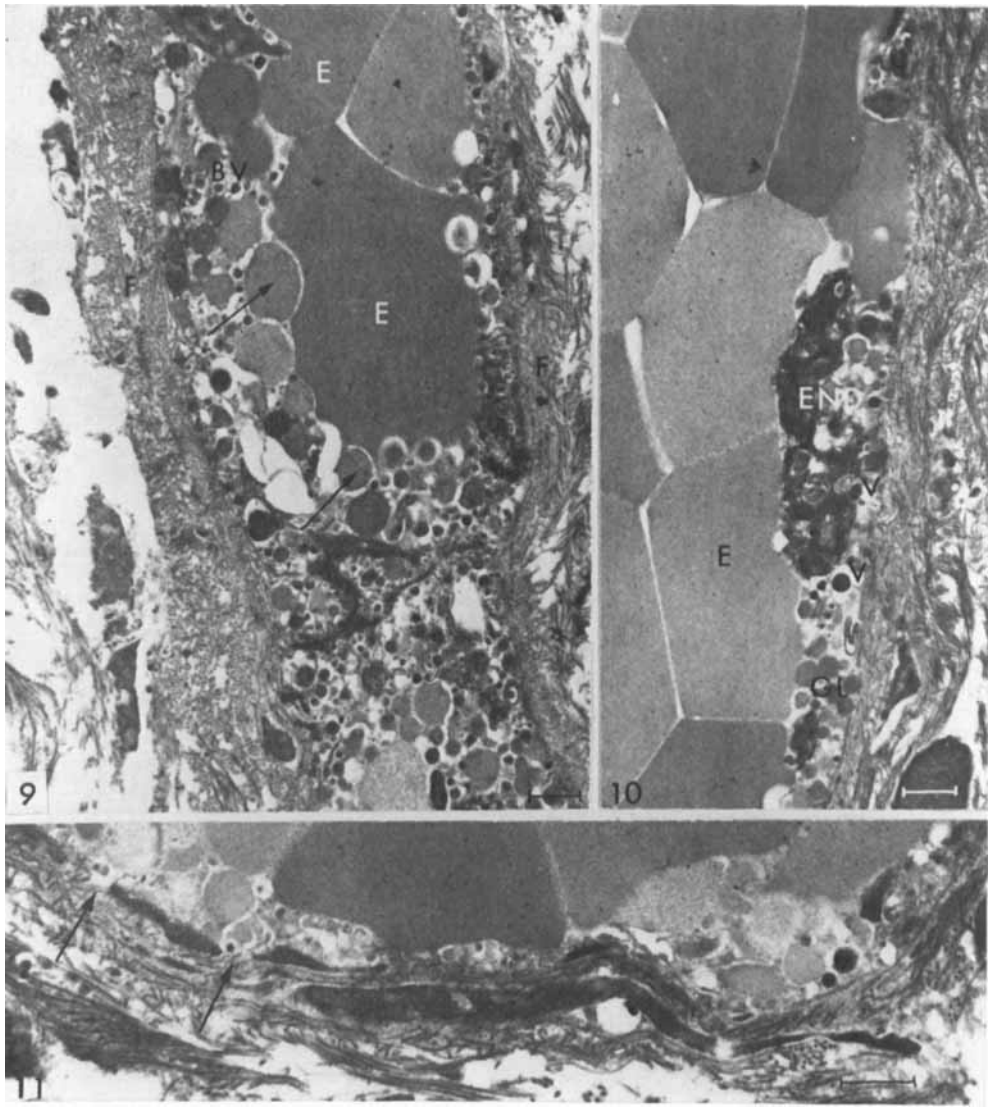
Figs. 3—5. Electron micrographs of the marginal area of the periodontal ligament. Fig. 3. Untreated material. Fibroblasts with rough-surfaced. endoplasmic reticulum, ER, filled with floccular material. × 11.000, bar = 1 μm. Fig. 4. Pressure zone. Increase of spaces, S, devoid of formed elements indicate oedema. N, cellular nuclei. Force 70 g. Duration 2 days. × 9.000, bar = 1 μm. Fig. 5. Cellular swelling in pressure zone. Vacuoles, V, of varying size, with or devoid of formed structures and dark globuli, GL, are seen in cytoplasm. Force 70 g. Duration 2 days. × 9.000, bar = 1 μm.



Figs. 6—8. Cellular changes in pressure zones. Force 70 g. Duration 2 days. Fig. 6. Cell nucleus, N, with scant remnants of cytoplasm surrounded by collagen fibrils, F, with characteristic pattern of cross banding inserting into cementum, C. × 11.000, bar = 1 μm. Fig. 7. Cell with partial loss of cytoplasm. Shrinkage of nucleus, N, containing aggregation of chromatin, CH, and empty interchromatic spaces. Point of rupture of the perinuclear envelope, arrow. × 9.000, bar = 1 μm. Fig. 8. Cell nucleus, N, containing a flocculent material. × 9.000, bar = 1 μm.

Periodontal ligament fibrils

2 days. Apart from a slight decrease of the interfibrillar spaces between the fibrils which constitute the connective tissue attachment to cementum, no alterations of the fiber system near the tooth were observed. The examination of root and adjacent soft tissue revealed intact collagen

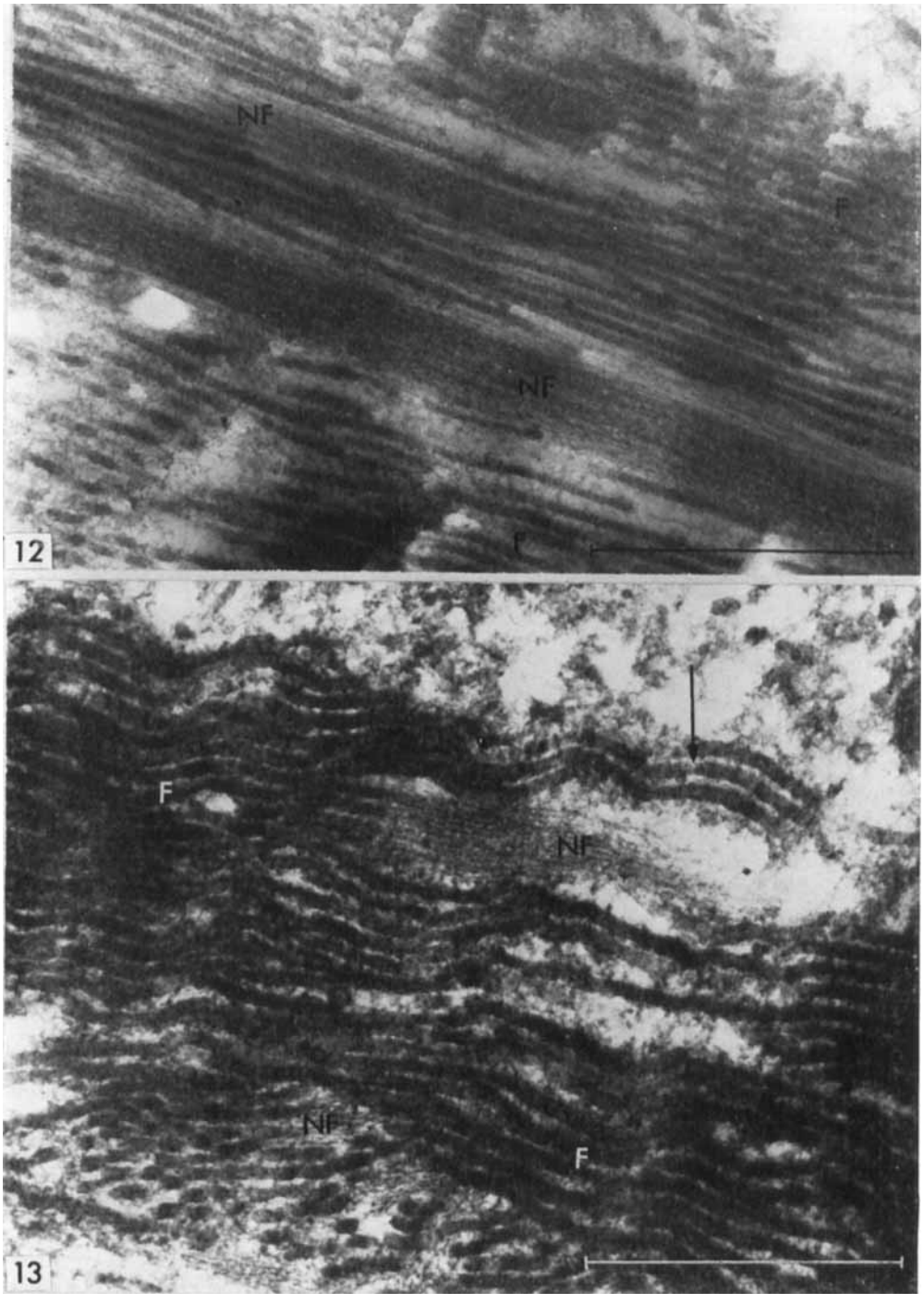


Figs. 9—11. Vascular changes in pressure zones. Force 70 g. Duration 2 days. Fig. 9. Erythrocytes, E, bordered by round structures, arrows, in blood vessel, BV, surrounded by fibrils, F. × 6 000, bar = 1 μm. Fig. 10. Blood vessel packed with erythrocytes, E, and bordered by swollen endothelial cells, END, containing globuli, GL, and vacuoles, V. × 6.000, bar = 1 μm. Fig. 11. Disintegration of vessel wall with communication between the lumen and perivascular structures, × 9 000, bar = 1 μm.

fibrils which could be traced into the cementum as they inserted at varying angles.

The diameter of the fibrils as well as the characteristic cross banding remained unchanged throughout the compressed zone (Fig. 6).

At some distance from the hard tissue, localised areas devoid of formed elements were observed between groups of fibrils. In these areas of looser texture, the interfibrillar spaces appeared to have increased (Fig. 4). In areas consisting of dense fibril-



Figs. 12—13. Fibrillar reactions in pressure zones. Force 70 g. Duration 21 days. Fig. 12. Bundles of non-striated filaments, NF, between collagen fibrils, F. × 41.000, bar = 1 μm. Fig. 13. Collagen fibrils, F, having split longitudinally into filaments, NF. Note increase of interfibrillar spaces, arrow, and irregular contour of collagen fibrils. × 41 000, bar = 1 μm.

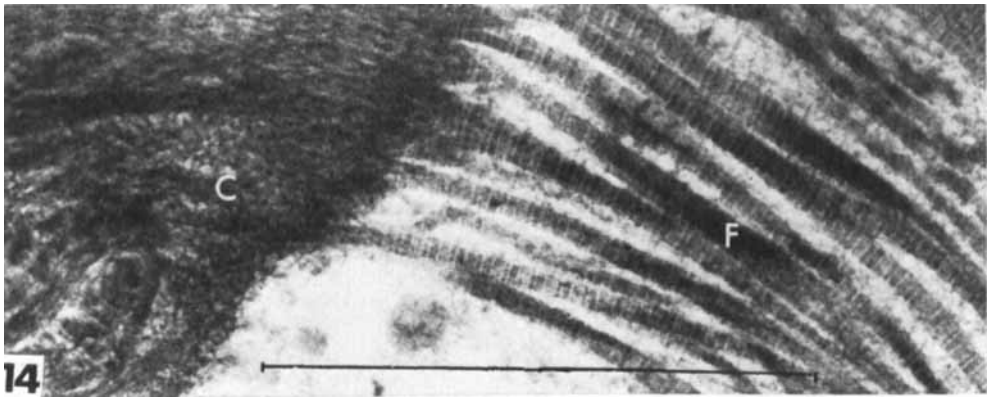


Fig. 14. Collagen fibrils, F, in hyalinized zone inserting into the cementum, C. Force 70 g. Duration 21 days. × 60.000, bar = 1 μm.

lar tissue, interfibrillar spaces were decreased (Fig. 6).

21 days. When the collagen fibrils were observed at low magnifications in the electron microscope, there were two characteristic features: a marked packing of fibrils and loss of the functional orientation of many fibril groups.

Groups of fine filaments could be observed between the collagen fibrils. The width of these groups varied between 0.1 and 0.25 μm. The individual filaments were less than 100 Å wide and did not reveal any indications of the striation pattern that is characteristic of collagen fibrils (Fig. 12). Some sections, however, contained cross-striated collagen fibrils which exhibited frayed ends which were split longitudinally into parallel filaments without striations (Fig. 13). In these sections the interfibrillar space had increased, and some of the individual fibrils appeared to have irregular contrours.

The fibrils inserting into cementum and bone seemed to be intact as judged by the uninterrupted cross-banding pattern (Fig. 14).

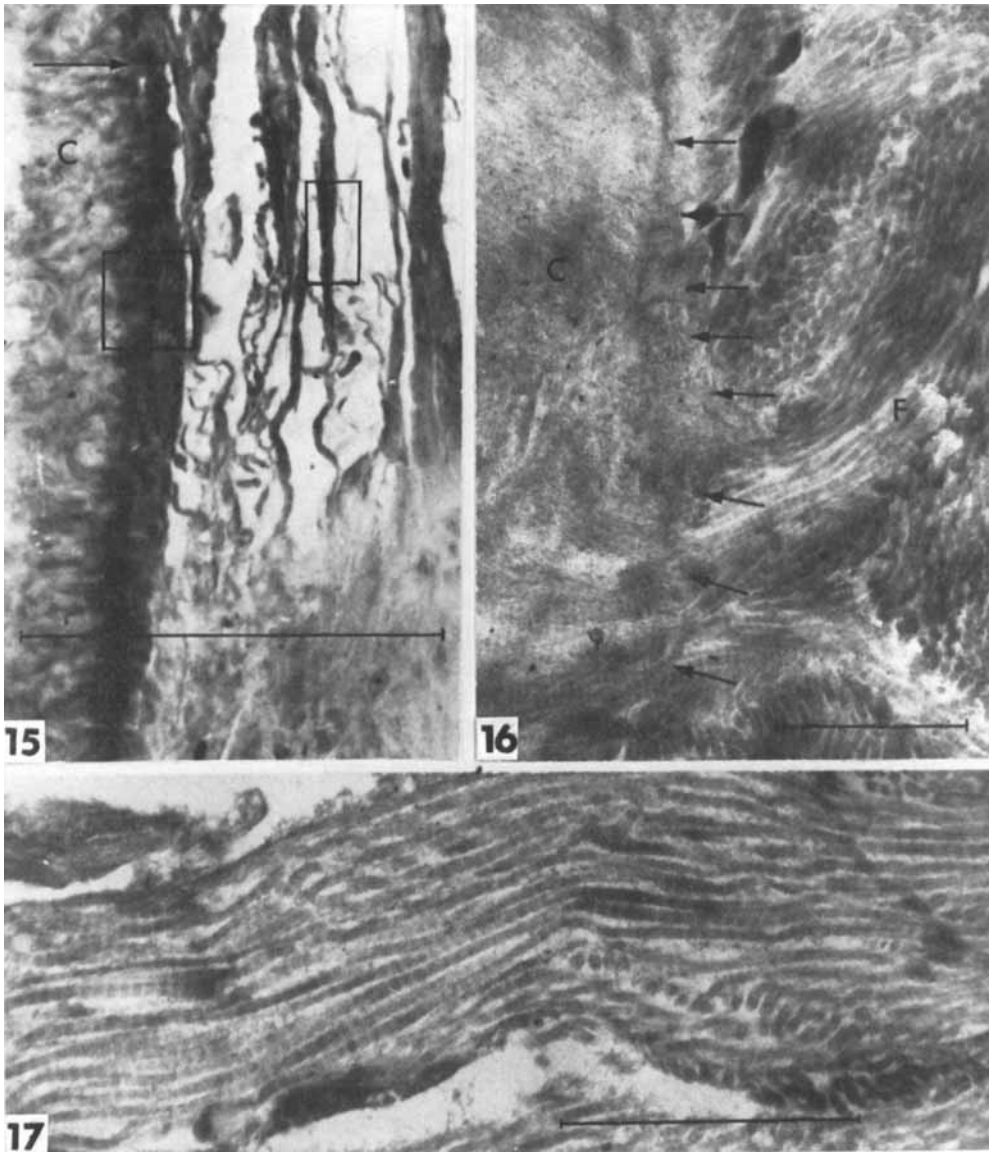
50 days. The fibrils immediately adjacent to the tooth surface had retained their cross striations and remained unbroken at their points of insertion into the cementum. Also in the long strands in the middle of the periodontal ligament the collagen fibrils revealed the characteristic striation pattern. The occurrence of fibrillar splitting did not seem to be higher than in the hyalinized zones of 21 days duration (Figs. 16, 17).

Alveolar bone

The osteocytes of the alveolar bone immediately adjacent to the compressed periodontal ligament appeared unchanged. The cell nuclei seemed to be intact with a regular contour without signs of shrinkage or aggregation of chromatin. In the surrounding bone the crossbanding of the fibrils was clearly discernible (Fig. 18).

DISCUSSION

The present light microscopic findings are in agreement with previous reports of orthodontic pressure zones in human experimental material (*Reitan*, 1951, 1954, 1962, 1969). Thus the specimens studied seem to be representative of the three experimental periods which were selected.



Figs. 15—17. Pressure zone in the marginal area of the buccal side of first premolar. Duration 50 days. Force 100 g. Arrow, direction of force. Fig. 15. Adjacent to cementum, C, a layer of compressed fibrils persist, while vertical strands of fibrils are seen more distant from the tooth surface. Razor blade section, toluidine blue. × 500, bar = 100 μm. Fig. 16. Boxed area (square) from fig. 15. Collagen fibrils, F, insert into the cementum, C. Arrows indicate the cementum surface. Electron micrograph × 23 000, bar = 1 μm. Fig. 17. Boxed area (rectangular) from fig. 15. Collagen fibrils with cross-striations. Electron micrograph × 35.000, bar = 1 μm.

However, the limited number of specimens and the fact that only 3 experimental periods are represented indicate some caution in the interpretation of the ultrastructural observations.

Perfusion procedures could obviously not be used for fixation, and an optimal reduction of the specimen size could not be performed if the whole width of the periodontal ligament, the adjacent ce-

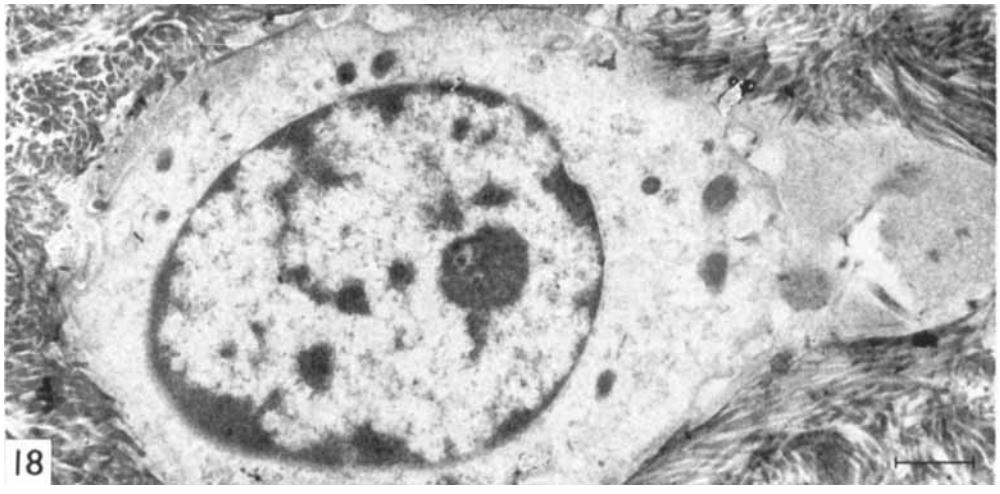


Fig. 18. Osteocyte in alveolar bone immediately adjacent to a hyalinized zone of the periodontal ligament. Force 120 g. Duration 21 days. × 10.000, bar = 1 μm.

mentum and alveolar bone was to be included. The resulting reduction in the quality of the micrographs due to incomplete fixation of the deep portions of the specimens was thought to be balanced by the possibilities of ascertained identification and simultaneous observations of all tissues involved.

The present study demonstrated extensive alterations of cells, vasculature and fibrils in the hyalinized zones of the periodontal ligament. The findings indicate that advanced cellular and vascular changes precede the fibrillar alterations. The results are in agreement with previous observations on necrosis of mouse liver, which have shown that while in the light microscope histologic alterations indicating cell death are most extensive in the nucleus, changes involving the cytoplasm actually precede nuclear alterations (*Trump, Goldblatt & Stowell*, 1965; *Magee* 1966).

Apart from differences related to the time factor, there was close agreement between the results in the present study and the results of studies in rats (*Rygh*, 1972a, 1972b).

In the present study even the short experimental period of 2 days was too long to reveal the initial cellular and vascular reactions. Thus the observations failed to demonstrate the earliest cellular reactions of the periodontal membrane to orthodontic pressure, as previously reported (*Rygh*, 1972a, 1972b), i.e. dilation of the endoplasmic reticulum and the perinuclear membrane with a general swelling and incipient degeneration of mitochondria, in the connective tissue cells as well as in the endothelial cells of the blood vessel walls.

It is generally held that cells die long before morphologic alterations develop which can be recognized as cell death (*Baker*, 1956, *Magee*, 1966, *Majno, La Gatutta & Thompson*, 1960). However, loss of granules, swelling of endoplasmic reticulum and mitochondria may be regarded as reversible processes, provided the cells deprived of oxygen are placed in a favorable milieu (*Baker*, 1956). In the present experiments the cells of the compressed areas had passed the point of irreversible degradation after 2 days,

as recognized by the observation of advanced swelling and vacuolization of the cytoplasm and transformation of mitochondria into empty shells.

The majority of cells were reduced to isolated, naked nuclei after 2 days. Dissolution and breakdown of the nuclear components was a slow process as indicated by the occurrence of nuclear remnants after an experimental period of 50 days. In the present study various forms of nuclear decomposition were observed, representing both karyolysis and karyorrhexis.

Generally, the ultrastructural vascular changes observed in this study are in agreement with findings made in rats (*Rygh*, 1972a; 1972b). Advanced swelling of endothelial cells with subsequent breakdown of the vessel walls, permitting communication between blood vessels and surrounding structures, generally observed after pressure had been exerted for 2 days in humans, was seen after 2 hours in rats. However, occurrence of intra- and extravascular crystallization of erythrocytes, as reported previously (*Rygh & Selvig*, 1973), was not observed in the present experiments. These crystals, thought to be crystalline hemoglobin, were formed in rats under the special circumstances of pressure and hemostasis which existed in the periodontal ligament as a result of the experimental procedures. The reason why crystallization has only been observed in rats may be the limited number of human specimens examined so far or else a possible difference between rat and human hemoglobin. It has even been suggested that the rat erythrocyte is in a unusual metastable state approaching that of incipient crystallization (*Drabkin*, 1945; *Ponder*, 1946; *Drabkin*, 1966; *Lessin*, 1968).

It is hypothesized that the round and irregular bodies at the periphery of the erythrocytes within the compressed blood vessels represent an incipient process of the breakdown of these cells. In the periodontal ligament of rat, at a certain stage of disintegration, many blood vessels contained masses of similar bodies (*Rygh*, 1972a).

In the light microscope, collagen fibers in hyalinized zones tend to become masked and confluent with the surrounding ground substance (*Reitan*, 1969). In the electron microscope, on the other hand, the collagen fibrils could easily be discerned between remnants of cellular and vascular structure. Although densely packed and apparently disoriented, a large majority of the fibrils revealed the cross-banding characteristic of collagen. The observed splitting of the fibrils into nonstriated filaments and increased occurrence of bundles of such microsfibrils is therefore interesting.

Collagen fibers in the periodontal ligament are composed of densely packed collagen fibrils with a diameter of 500—800 Å, and a characteristic cross-banding with a periodicity of about 640 Å. In the presence of chronic inflammation these fibrils become less densely packed and bundles of thin fibrils or filaments appear instead. Furthermore, fibrils disintegrate longitudinally into filaments without periodicity (*Selvig*, 1966). Similar changes have been observed in the hyalinized zones of rats (*Rygh*, 1973). Such alterations have also been observed under different pathological conditions and probably represent a non-specific connective tissue response to various non-physiological agents (*Selvig*, 1966).

The time factor, i.e. the duration of the hyalinized zone, seems to be of importance with regard to the degree of splitting of fibrils. This reactions was not seen after force application for 2 days. Even

after 21 days the number of fibrils which had disintegrated into filaments, consisted of less than 10 per cent of the area in the electron micrographs. It is not as yet known how far the process of dissolution of the connective fibrils will proceed if the stage of regeneration is postponed for a considerable time.

Although one cannot exclude the possibility that patterns of degradation exist, other than longitudinal splitting, the observations revealing, that most fibrils retain their cross banding and continuity with the cementum in hyalinized zones of a 50 days experimental period, indicate that the individual fibrils degrade slowly. This is not in agreement with recent observations in the scanning electron microscope by *Kvam*, (1973), indicating loss of periodic cross striations and structural organization of the fiber bundles inserting into cementum in hyalinized zones after experimental periods of only 10 days. The discrepancy may be explained by differences in the methods of specimen handling and examination.

Since experimentally produced primary hyalinized zones in humans very seldom remain longer than three weeks (*Reitan*, 1969), it seems likely that until the hyalinized tissues are removed, the continuity between fibrils and cementum is not disrupted during clinical or orthodontic procedures, except possibly in localized foci of heavy pressure and where hyalinization is prolonged.

The osteocytes of the alveolar bone adjacent to the compressed periodontal ligament revealed no alterations indicating degeneration or cell death or necrosis of the bone tissue. These findings are not in agreement with those of *Oppenheim*, (1944), *Macapanpan et al.*, (1954) and *Picton*, (1969), but support previous observations in rats by *Rygh*, (1972b). The different observations may, however, possibly be explained by the amount of the applied experimental force.

It seems justified to conclude that when the orthodontic forces applied to human teeth are kept within the range presently used in clinical practice, degeneration and necrosis is limited to circumscribed areas of the periodontal ligament.

The structural alterations which were observed systematically in the rat material, supplement the relatively sparse human material. On this basis it is now possible to form a coherent concept of the ultrastructural alterations in connection with hyalinization of the periodontal ligament which also applies to the reactions in humans.

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