Acta odont scand 1972 *30:* 575—593

Keywords:
Periodontium
Rats
Orthodontics
Microscopy, electron

From:
The Research Laboratories and
Department of Orthodontics,
School of Dentistry,
University of Bergen,
Bergen, Norway

ULTRASTRUCTURAL CELLULAR REACTIONS IN PRESSURE ZONES OF RAT MOLAR PERIODONTIUM INCIDENT TO ORTHODONTIC TOOTH MOVEMENT

PER RYGH

Changes in the cells of the periodontal ligament of teeth subjected to orthodontic forces have been described. The purpose of the present study was to characterize these changes at the ultrastructural level. In 67 rats, maxillary first molars were moved buccally by means of fixed appliances exerting forces of 5—25 g. Animals were sacrificed after periods of 30 min to 28 days. The experimental teeth with surrounding periodontal tissues were removed and processed for electron microscopy. Cellular changes not involving the nucleus, such as dilation of the endoplasmic reticulum and moderate swelling of mitochondria occurred within 30 min of force application. After 2 hrs, this cellular swelling was marked. Large vacuoles had formed in the cytoplasm, and the mitochondria frequently revealed considerable enlargement. More advanced degradation of cellular components was observed after experimental periods of 2 hrs or more. Rupture of the cell membrane had frequently occurred leaving isolated nuclei in various stages of decomposition.

It seems that hyalinization involves severe injuries of fibroblasts and other connective tissue cells, resulting in cell death. However, necrosis was limited to circumscribed areas in the periodontal ligament. Dispersed globuli and nuclear fragments were occasionally observed within hyalinized zones at 2—7 days. Regenerative processes were prevailing in the 7—28 days specimens.

The sequence of changes which occur in the periodontal ligament of teeth subjected to orthodontic forces has been studied extensively with the light microscope. Seventy years ago *Sandstedt* (1940, 1905) observed development of cell-free, structureless zones on the pressure side, areas he described as sclerotic. The observation that these zones appear as a glasslike, confluent mass, resembling hyaline cartilage in hematoxylin eosin-stained sections, has led to the use of the term hyalinization of periodontal tissues (*Schwarz*, 1932; *Skillen & Reitan*, 1940; *Oppenheim*, 1942; *Macapanpan, Weinmann & Brodie*, 1954). Similar cell-free zones may even occur during physiological migration of teeth (*Kronfeld & Weinmann*, 1940).

Received for publication, June 14, 1972.

According to the previous studies, the first indication of hyalinization consists in increased basophilia and a shrinkage of cell nuclei, pyknosis, as compressed collagen fibers gradually unite into a more or less cell-free mass. The fibers tend to become masked and confluent with the surrounding ground substance (*Reitan*, 1969).

Apart from recent reports on ultrastructural vascular changes in hyalinized zones (*Rygh*, 1972; *Rygh & Selvig*, 1973), the electron microscope does not seem to have been applied to the study of tissue reactions incident to orthodontic tooth movement.

The observation that a certain degree of hyalinization must be expected in all clinical orthodontic work (*Reitan*, 1969), dependent upon anatomical characteristics and magnitude of force, indicates that a further analysis of the development and fine structure of hyalinized zones might improve the understanding of tissue reactions incident to tooth movement.

The purpose of the present investigation was to characterize at the ultrastructural level the cellular changes in orthodontic pressure zones, to establish whether a particular pattern of cellular response exists, and to determine the ultimate fate of the altered connective tissue cells.

During the process of hyalinization two separate phases can be distinguished: 1) an initial period, characterized by cellular degradation and masking of fibers, and 2) a secondary period, characterized by repair and restitution. The present report deals with the initial period.

The rat was chosen as the experimental model, as this animal has proved to be well suited for studies of tooth movement. Because of the minute dimensions, thin sections which included the root surface, the periodontal ligament and the alveolar bone could be studied in the electron microscope, thus securing orientation within the specimen.

A group of untreated animals was also included in the study. Reference to the normal rat molar periodontium is made when necessary for comparison.

MATERIAL AND METHODS

Experimental tooth movement was carried out in 67 Wistar rats of both sexes. A control group of 11 animals without any experimental appliance was also included. The age of the animals at the start of the experiment was 84 $\pm$ 15 d (mean $\pm$ s.d.) and the weight 185 $\pm$ 38 g.

In the experimental animals, one maxillary first molar was moved buccally with a fixed appliance, as described previously (*Rygh*, 1972). The appliance

was activated to provide a constant force of 5, 10 or 25 g. The experimental periods were 30 min, 2, 6, 12, 24, 48, 60 hrs, 5, 7, 14 and 28 days.

The 10 g series consisted of three animals in each experimental group, apart from the 30 min, 2, 12, 24 hrs group that consisted of 6 animals. The 5 and 25 g series included one animal at each time period.

At the end of the experimental period, each experimental tooth with adjacent periodontal tissues was dissected out under ether anesthesia. Tissues from one series of eleven animals subjected to an experimental force of 10 g was fixed in 10 % buffered formalin, dehydrated in ethyl alcohol, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

All other specimens were fixed in 3 % glutaraldehyde for 2 hrs. rinsed for 30 min in a 0.1 M sodium cacodylate buffer at pH 7.2, to which had been added 0.2 M sucrose, and demineralized in 0.25 M disodium ethylenediaminetetraacetate for 5 days. After postfixation in 1 % phosphate-buffered osmium tetroxide, the specimens were dehydrated in acetone and embedded in Durcupan® * in gelatin capsules.

For orientation, sections were cut with a razor blade and stained with 0.1 % aqueous toluidine blue at pH 11. The blocks were then trimmed to include the marginal area on the buccal side of the tooth. Thin sections, 300—400 Å in thickness, were cut with glass knives on an LKB ultramicrotome. The grids were floated on a saturated solution of uranyl acetate in 50 % ethyl alcohol and double-stained with lead citrate (*Reynolds*, 1963). Sections were examined in an electron microscope (Philips EM 300), operated at 80 kV.

RESULTS

Light microscopy

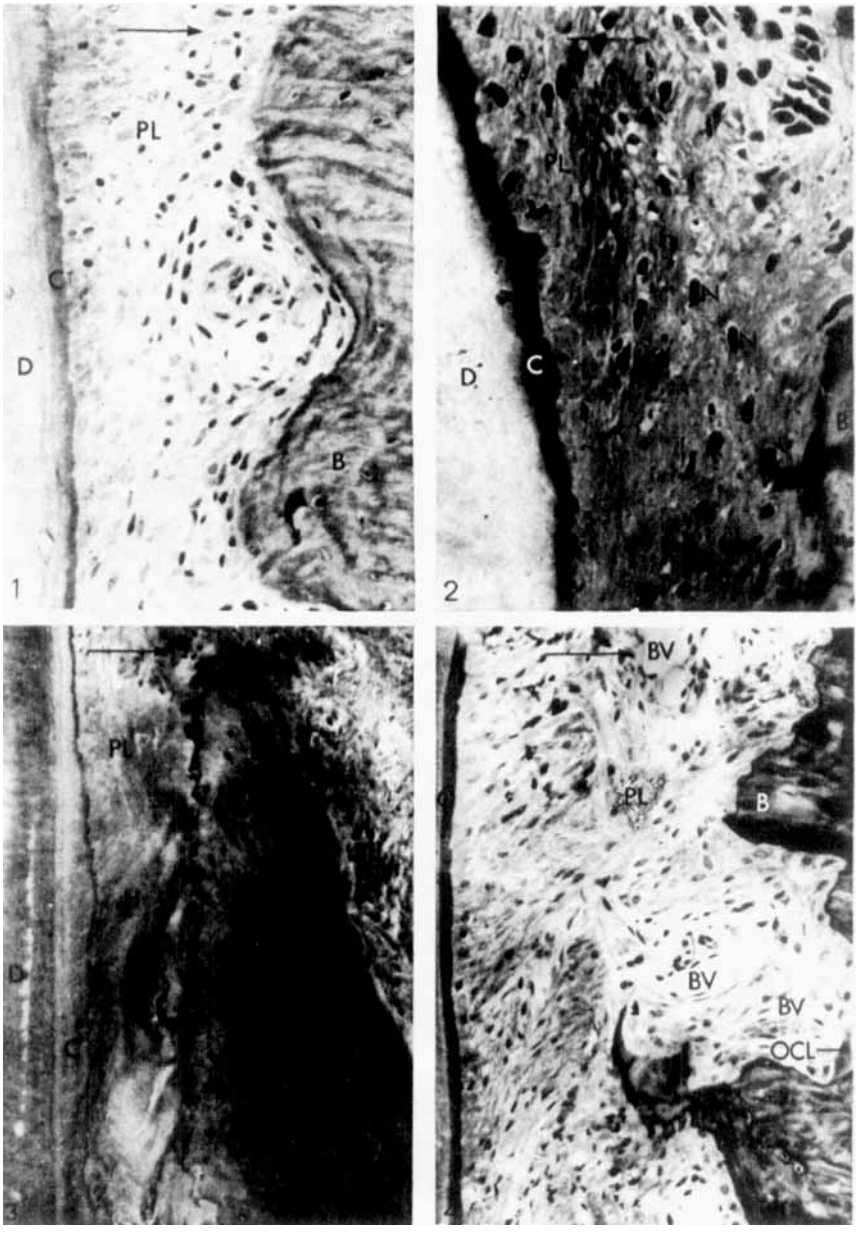
A description of the findings with special reference to vascular reactions has been presented earlier (*Rygh*, 1972). Presently, the cellular changes in the connective tissue will be briefly reported.

After 30 min application of a 10 g force the periodontal ligament in the marginal area appeared compressed. Cellular changes were not observed (Fig. 1).

After 2 hrs more marked deviations from normal structure were observed. Some cells revealed pyknotic nuclei. Other cells appeared without visible cytoplasm as isolated nuclei (Fig. 2).

---

* Fluka A. G., Buchs, Switzerland.



Figs. 1—4. Pressure zones in the marginal area of the periodontal ligament (PL) on the buccal side of first rat molars. Force 10 g. Razor blade sections, stained with toluidine blue. B, alveolar bone. C, cementum. D, dentin. Arrow, direction of force. Fig. 1. Slight compression of the periodontal ligament without visible cellular changes. Duration 30 min. × 300. Fig. 2. Pyknosis of cell-nuclei (N) and reduction of cytoplasm in the narrow, compressed parts of the periodontal ligament. Duration 2 hrs. × 560. Fig. 3. Area of extensive hyalinization (H) almost devoid of cellular elements. × 220. Fig. 4. Reparative stage with proliferation of vascular (BV) and cellular elements and direct resorption by osteoclasts (OCL). × 300.

In the period from 6 to 12 hrs the changes were more extensive. Localized areas seemed to be cell-free and had a homogenous appearance.

After 24 hrs the alterations were more advanced. A cell-free mass had developed adjacent to bone spicules, while the cells in bone clefts and in curvatures of the tooth surface seemed to be well preserved.

Within 2—3 days the pressure zone was almost devoid of cells. Naked nuclei were occasionally observed (Fig. 3).

After 5—7 days the compressed regions appeared to be in a state of repair, characterized by proliferation and ingrowth of vascular and cellular elements (Fig. 4).

A similar sequence of alterations was seen in all specimens subjected to experimental tooth movement. However, the pressue zones were progressively more extensive with increasing experimental force.

Electron microscopy

The earliest tissue changes were seen in limited areas, which appeared to have been under relatively strong pressure.

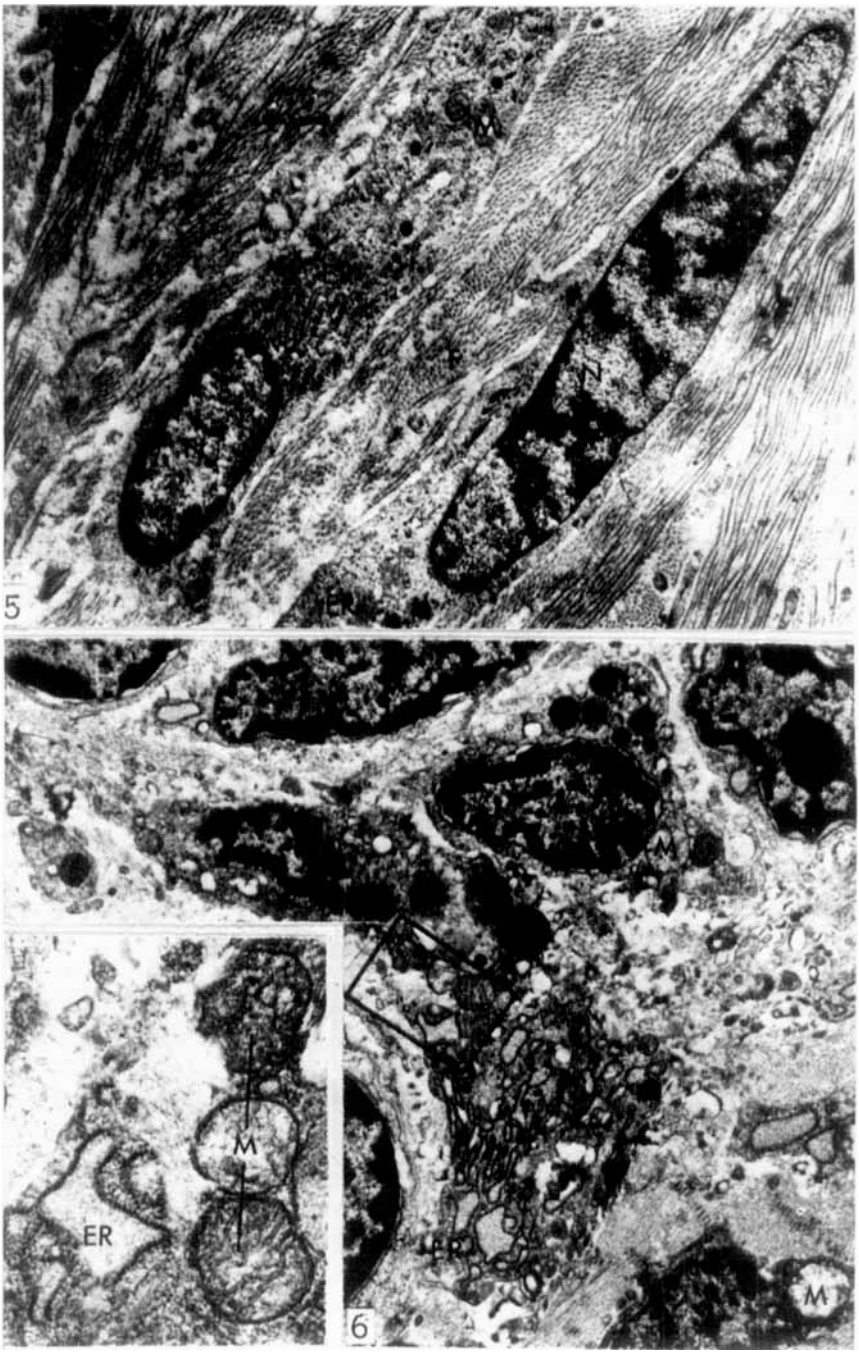
At the ultrastructural level, no particular cell reactions were characteristic of a certain experimental period. Within the small, local pressure foci some cells had undergone extensive changes, while cells in adjacent but more protected locations revealed less alterations.

Cellular reactions seemed to follow a relatively consistent pattern. Similar reactions were observed in osteoblasts, cementoblasts and fibroblasts. The following stages could be distinguished:

I. early cellular changes, not involving the nucleus,
II. extensive cellular alterations,
III. disintegration of cells and loss of cell identity.

I. Early cellular changes, not involving the nucleus. In specimens from untreated animals; osteoblasts, fibroblasts and cementoblasts contained a well developed rough-surfaced endoplasmic reticulum that appeared as flattened or moderately dilated cisternae, filled with a floccular material (Fig. 5).

Dilation of the endoplasmic reticulum seemed to be an early reaction to orthodontic pressure. The degree of dilation varied. A mere widening of the endoplasmic sacs was seen in some cells (Fig. 6), while single distended cisternae containing a stainable substance dominated the entire cytoplasm of other cells (Fig. 7). Also the perinuclear envelope was wider than in the control material (Fig. 8).


N
ER
5
M
M
ER
ER
M
6

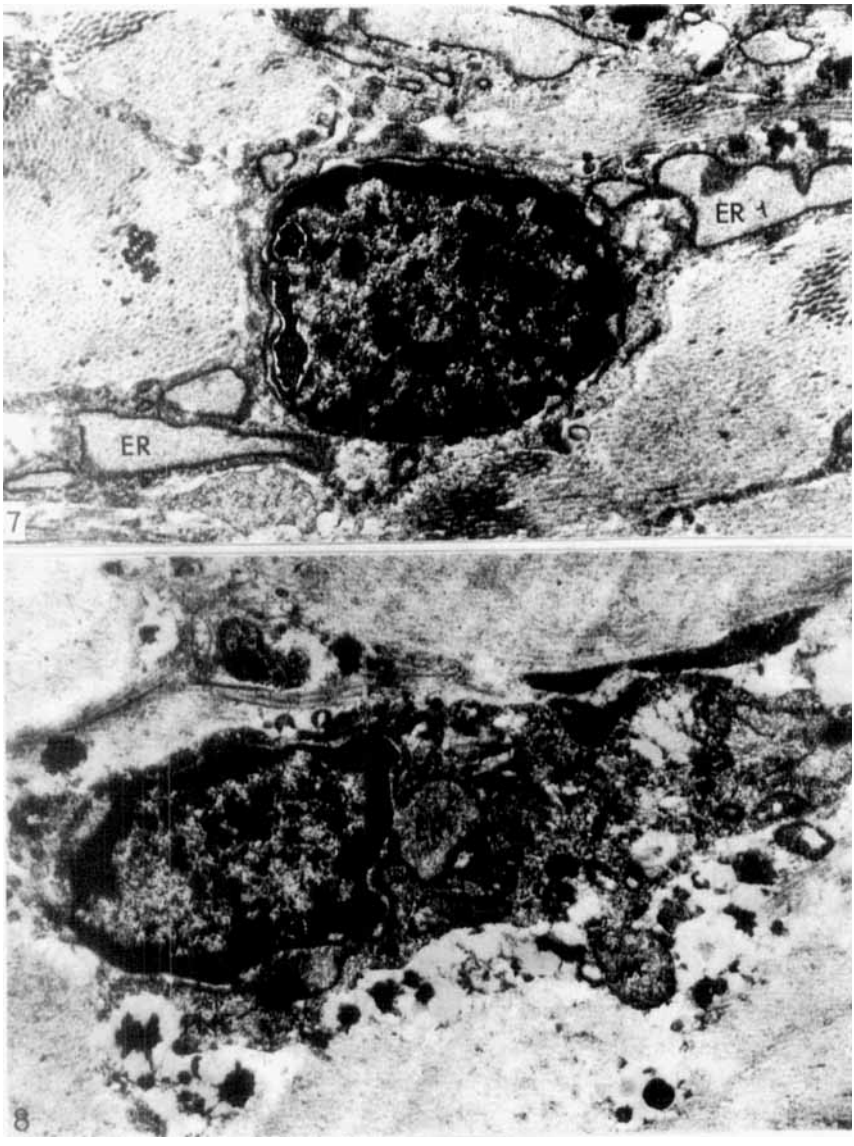


Fig. 7. Electron micrograph showing extensive dilation of the endoplasmic reticulum (ER). Force 10 g. Duration 30 min. × 15,000. Fig. 8. Dilation of endoplasmic reticulum (ER) confluent with the perinuclear envelope (PNE). M, mitochondrion. Force 10 g. Duration 2 hrs. × 15,000.

←

Figs. 5–6. Electron micrographs from the marginal area of the periodontal ligament on the buccal side of rat molars. Fig. 5. Normal appearance of fibroblasts and collagen fibers in periodontal ligament. ER, endoplasmic reticulum. F, fibrils. N, cell nucleus. M, mitochondrion. × 10.000. Fig. 6. Moderate dilation of the endoplasmic reticulum (ER) and swelling of mitochondria (M). Force 10 g. Duration 30 min. × 10.000. Inset: Boxed area from fig. 6 showing intact and altered mitochondria. × 30,000.

The mitochondria usually appeared round or oval in shape with distinct outer and inner membranes in cells of the periodontal ligament from untreated animals. The cristae mitochondriales usually extended beyond the midline. An early reaction to orthodontic pressure was a general swelling of the mitochondria (Fig. 6).

These changes were seen after application of an orthodontic force of 10 g for 30 min. Similar changes were also seen at later time points, but in cells at some distance from the zone of maximal compression.

II. Extensive cellular alterations. More advanced stages of cellular changes were observed in sections from experimental periods of 2 hrs or more. However, varying with localization, minor and severe forms of cellular degradation were seen. The extracellular milieu in the zone of maximal compression was characterized by edema, as indicated by an increased occurrence of clear spaces devoid of formed elements (Figs. 9, 14). Cellular swelling was also prominent, with marked dilation of the endoplasmic reticulum. Swelling of the mitochondria was a consistent finding.

In some cells, swollen mitochondria were observed adjacent to intact mitochondria (Fig. 9). These organelles appeared to decrease in number and increase in size. The contents of the mitochondria appeared less electron dense in the swollen than in the intact mitochondria. The cristae mitochondriales were often separated and distorted, and at later stages they completely disappeared. The outer membranes could usually be distinguished, even in mitochondria with marked swelling of the matrix and advanced disorganization of the cristae (Fig. 9).

In some instances, the impression was gained that swollen mitochondria were being released from the cell. At this stage mitochondria were often observed in close contact with both nucleus and plasma membrane (Figs. 10, 11).

The nuclear envelope, which in unaltered cells consisted of two electron-dense layers and a distance between the membranes measuring approximately 200 Å, appeared dilated after application of a 10 g force for 30 min (Fig. 7). However, in specimens of longer experimental periods, the two layers of the nuclear membrane were markedly separated in some areas and more advanced alterations of the nucleus were observed (Figs. 8, 11). The nucleus showed shrinkage, extensive invaginations of the nuclear membrane, and a relative increase of darkly stained chromatin (Figs. 12, 13).

III. Disintegration of cells and loss of cell identity. The appearance of the pressure zone varied. Areas composed mainly of cellular elements, i.e.

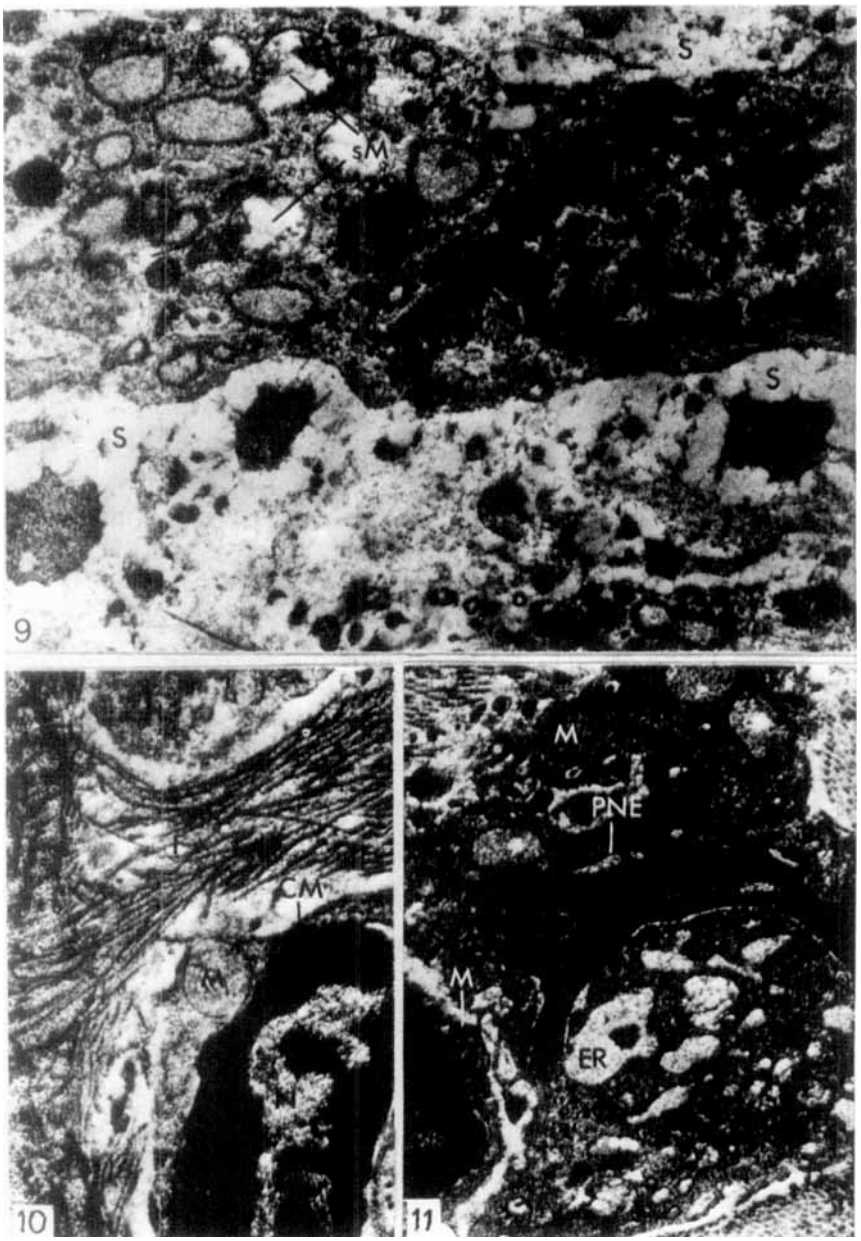
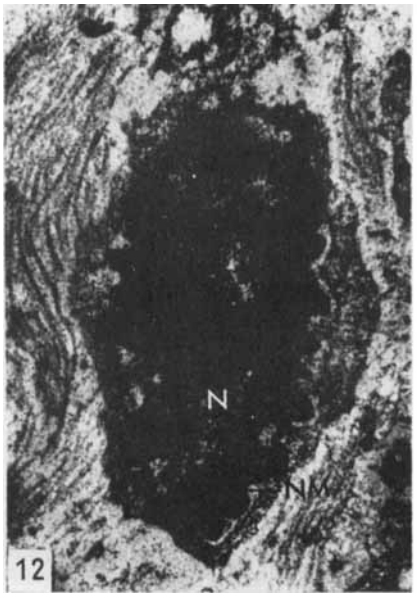


Fig. 9. Intact mitochondria (iM) and swollen mitochondria (sM) in the same cell. The swollen mitochondria show a decrease in relative density, and distortions and disappearance of the cristae mitochondriales (arrows). Increase of space (S) devoid of formed elements indicate edema. Force 15 g. Duration 2 hrs. Electron micrograph. × 15,000. Fig. 10. Cementoblast near cementum (C) and fibrils (F). Mitochondrion (M) in close contact with nucleus (N) and cellular membrane (CM) gives the impression of being released from the cell. Force 10 g. Duration 2 hrs. Electron micrograph. × 12,500. Fig. 11. Electron micrograph showing dilation of the perinuclear envelope (PNE) and endoplasmic reticulum (ER), and mitochondria (M) giving the impression of being released from the cell. Force 5 g. Duration 3 days. × 10,000.



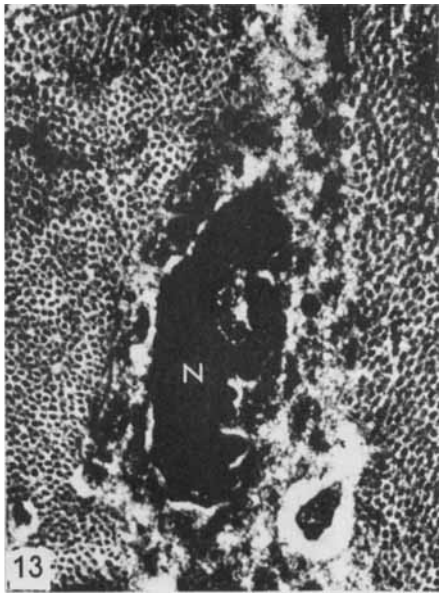


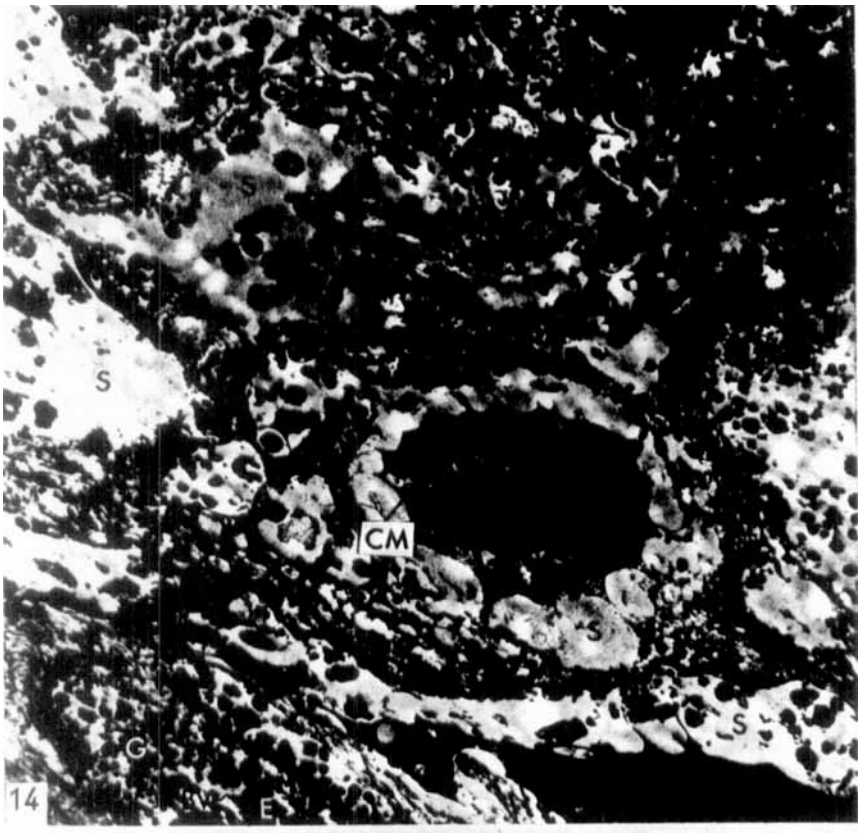
Fig. 12. Electron micrograph showing extensive invaginations of the nuclear membrane (NM) and shrinkage of cell nucleus (N). Force 10 g. Duration 3 days. × 16,000. Fig. 13. Electron micrograph showing advanced shrinking of cell nucleus (N) with increase of darkly stained chromatin. Force 10 g. Duration 2 hrs. × 18,000.

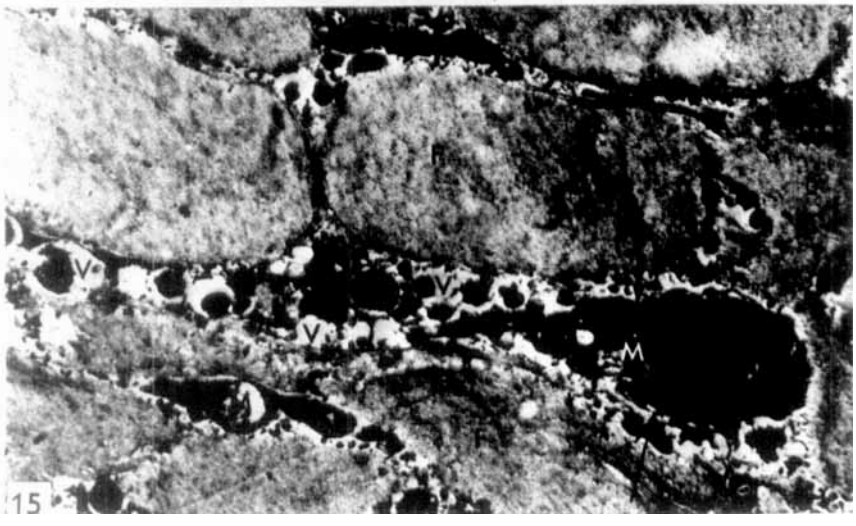
near blood vessels, were characterized by a separation of the formed elements. In such areas cells with well defined cytoplasmic membranes could still be observed after 3 days (Fig. 14).

In areas where cells were interspersed between densely packed fibers, intracellular swelling was more pronounced. Advanced degradation of cellular components was observed after experimental periods of 2 hrs or more. Mitochondria showed considerable enlargement and distortions of cristae. In some cells large vacuoles extended from the nucleus to the plasma membrane. Breakdown of the perinuclear envelope was observed near some vacuoles. In cells exhibiting considerable swelling, the surface membrane was not readily seen. The cytoplasm appeared filamentous or had disappeared.

→

Fig. 14. Hyalinized zone in loose tissues characterized by separation of the formed elements, increase of space (S), and cells with well defined membranes (CM). Blood vessel (BV) containing fragments of erythrocytes (E) and globuli (G). Force 10 g. Duration 3 days. Electron micrograph. × 6,000. Fig. 15. A cell, that is interspersed between densely packed fibrils (F), reveals advanced swelling with formation of vacuoles (V), degeneration of mitochondria (M), and partial breakdown of perinuclear envelope (arrows). Force 10 g. Duration 2 hrs. Electron micrograph. × 6,000.


S
S
CM
S
S
G
E
14


V
V
V
M
15

Dilated cisternae of the endoplasmic reticulum and membranes limiting degenerated mitochondria could be defined (Fig. 15).

The process of rupture of the cell membrane was not observed. However, at a certain stage only naked nuclei remained, surrounded by fibrillar elements or structures of a looser texture (Fig. 16). The dissolution or disintegration of the cytoplasmic membrane left the cellular components in the extracellular space. When cells were located between compressed fiber bundles, only scant remnants of the cytoplasmic components could be observed after as short experimental period such as 2 hrs. In areas of looser texture, components of degenerated connective tissue cells could not be distinguished from breakdown products of vascular structures.

The isolated nuclei revealed considerable variation of contour. Some nuclei had a wrinkled appearance while others had an even outer form. At this stage the whole nuclei were more heavily stained than the control material and as compared with those observed in earlier stages. The nuclear membrane could not be identified. There were indications of a dissolution of nuclear contents, and a release of nuclear components into the environment was observed. The released breakdown products had taken more stain than was seen in the parts still contained within the nucleus (Fig. 17).

This process of nuclear breakdown seemed to take place at varying rates. In regions of dense fibrillar structures, i.e. near the cementum, cellular elements were barely traceable after 24 hrs of experimental pressure, while in regions of looser texture nuclear remnants and globuli from the cytoplasm remained interspersed between fibrillar elements (Fig. 18).

While cementoblasts, fibroblasts and osteoblasts in the compressed zones showed no difference in rate and pattern of degradation, cells situated in bays or curvatures between bone spicules were better preserved and exhibited a slower rate of degradation than cells in less protected parts of the periodontal ligament.

Osteocytes in lacunae of the buccal alveolar bone adjacent to hyalinized parts of the periodontal ligament revealed no changes in cell structure.

DISCUSSION

The present study demonstrated a progressive degradation leading to death of cells during development of the hyalinized zones in the periodontal ligament. The cellular breakdown could be followed through several stages with the ultimate disappearance of cellular structures.

The light microscopic observations were in accordance with previous

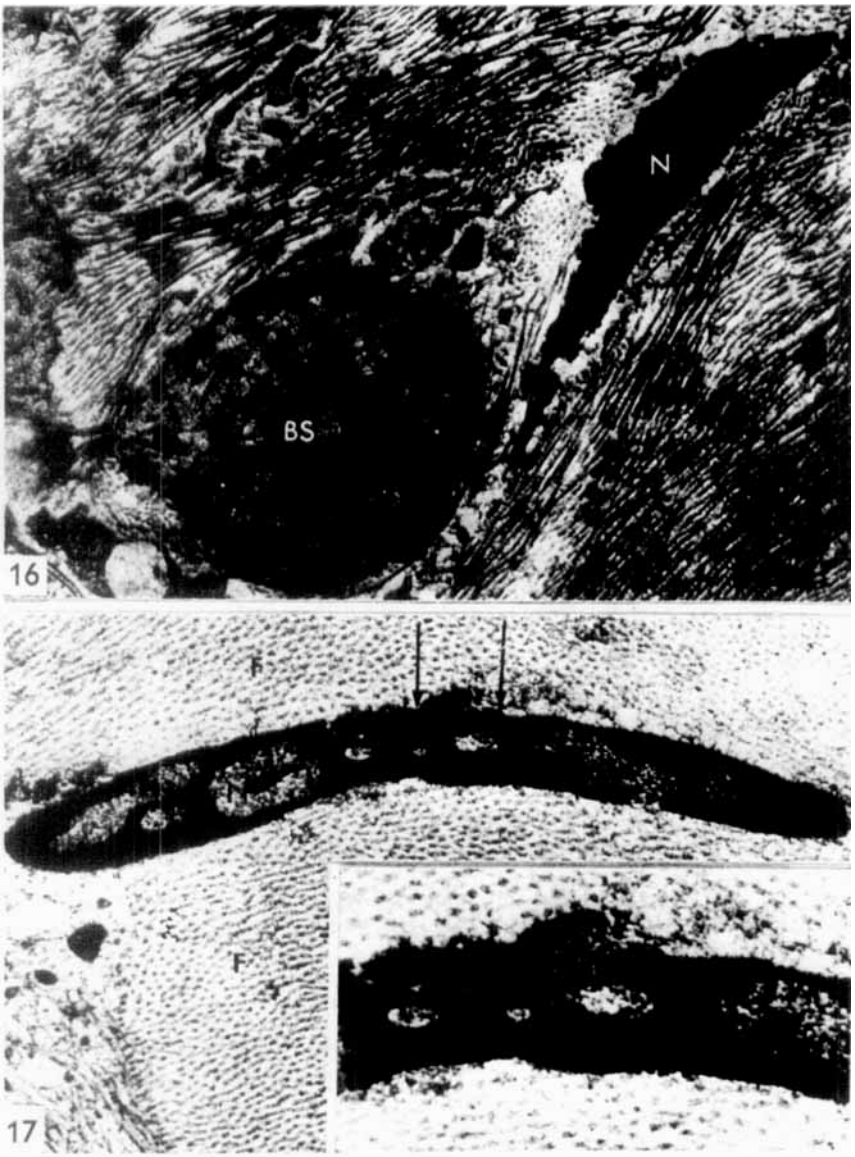


Fig. 16. Cell nucleus (N) without cytoplasm near bone surface (B) with bone spicule (BS). F, collagen fibrils. Force 20 g. Duration 2 hrs. Electron micrograph. × 15,000. Fig. 17. Cell nucleus (N) between collagen fibrils (F) showing dissolution of nuclear contents and release of darkly stained nuclear components (arrows) into the environment. Force 10 g. Duration 2 hrs. Electron micrograph. × 15,000. Inset: Boxed area from fig. 17. × 30,000.

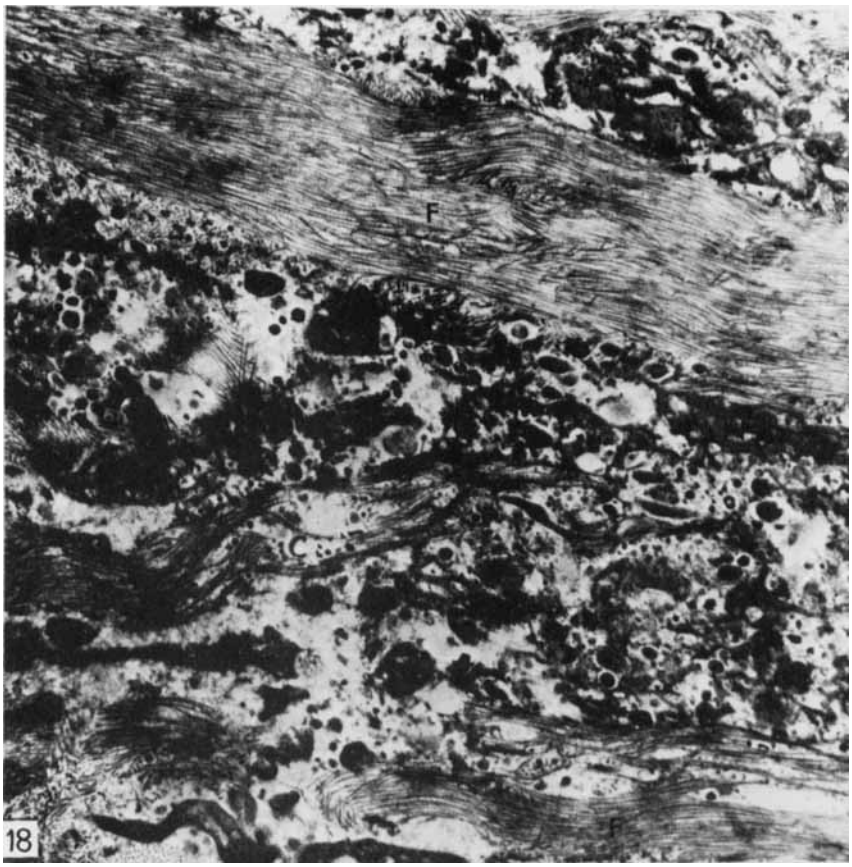


Fig. 18. Remnants from cell nuclei (N), globuli and unidentifiable cellular elements between collagen fibrils (F) in hyalinized zone. Force 10 g. Duration 24 hrs. Electron micrograph. × 9,000.

descriptions of cellular changes in pressure zones incident to orthodontic tooth movement. Rapid hyalinization is characteristic of the rat periodontal ligament (*Macapanpan et al.*, 1954; *Kvam*, 1969; *Azuma*, 1970), whereas in the human periodontium initial hyalinization occurs after 30—40 hrs (*Reitan*, 1951).

The absence of cellular changes after an experimental period of 30 min and presence of pyknotic nuclei after 2 hrs are in agreement with previous studies which have reported pyknotic cell nuclei after 1 h and complete hyalinization with total loss of fibroblasts after 24—48 hrs in the regions of greatest pressure (*Kvam*, 1967; *Reitan & Kvam*, 1971).

It is generally held that, at the level of the light microscope, histologic changes that indicate death of a cell are more prominent in the nucleus than in the cytoplasm (*Robbins,* 1967). The first indication that a cell is dying is increased basophilia and shrinkage of the nucleus. With the electron microscope, however, it has been found that mitochondrial changes actually precede nuclear alterations (*Trump, Goldblatt & Stowell,* 1965). This is supported by the present study which showed only cytoplasmic changes after 30 min.

Since experimentally induced cellular changes in the periodontal ligament do not seem to have been described, the present findings had to be compared to previous experimental studies which have dealt with the liver. It is generally held, however, that mammalian cells follow a rather consistent pattern of disorganisation and cell death, varying mainly with regard to the rate of degradation (*King, Paulson, Hannaford & Krebs,* 1959).

Moore & Brody (1960) produced ischemic necrosis by ligating the anterior mesenteric and celiac arteries and portal vein in the rat for a period of 2 hrs. Mitochondria from these livers had an increased tendency to swell and contained 50 per cent less protein than normal.

From in vitro studies of mouse liver undergoing autolysis, believed to represent changes in cells that are deprived of their blood supply, *Trump et al.* (1965) reported mitochondrial swelling with decrease in matrix density after 30 min. Later mitochondrial changes included vesiculation, distortion and fragmentations of membranes and aggregations of dense material in the matrix culminating at 24 hrs in the transformation of mitochondria into empty shells. The occurrence of mitochondrial swelling after experimental pressure for 30 min in the present study is in accordance with these findings. Also the later mitochondrial changes seen in the pressure zones showed a sequence of events similar to those reported in liver autolysis. However, while complete breakdown of mitochondria in liver cells appeared between 8 and 24 hrs (*Moore & Brody,* 1960), similar decomposition was seen after only 2 hrs in the periodontal ligament. In fact, after 2 hrs a rupture of the cellular membrane with release of the cytoplasmic components frequently had taken place in the present experiments.

According to *Magee* (1966), it is possible to trace various causes of cell necrosis to a severe interference with the utilization of oxygen by the cell, or to disruption of cellular structures. Thus, necrosis is considered to be caused either by ischemia or by physical or toxic injury. The primary attack in ischemia is related to lack of oxygen and other cellular nutrients. Physical trauma causes death of cells by rupture of cell membranes or by intracellular dislocations (*Robbins,* 1967).

It is therefore hypothesized that the considerable difference in time between degradation of cell organelles in autolysing ischemic liver cells and compressed cells in the periodontal ligament may be attributed to the physical trauma elicited by the experimental force application.

The cellular changes that were observed in the present study followed a relatively consistent pattern. The characteristic dilation of the endoplasmic reticulum is similar to changes in liver cells after injection of carbon tetrachloride (*Smucker, Iseri & Benditt,* 1962). It is generally held that changes in permeability properties of cellular membranes with an electrolyte and water shift are related to injury and necrosis. Potassium is lost from the cell concurrently with the entrance of sodium and water. There is also an inhibitory effect on protein synthesis.

Isolated nuclei in various stages of degradation after rupture of cellular membranes were regularly present in pressure zones after 2 hrs. This is in accordance with the earlier studies by light microscopy, where the changes were termed pyknosis of cells or of cell nuclei (*Macapanpan et al.*, 1954; *Kvam* 1967). The present investigation based on electron microscopy, provided more detailed information on the nuclear changes.

Although observations of single sections do not prove positively that the nuclei had actually become smaller, several indications of shrinkage of nuclei were presented. It is uncertain whether the initial dilation of the envelope reflects changes of the nucleus. A pronounced wrinkling of nuclear contour with formation of deep invaginations suggests that shrinkage of the nucleus had taken place. The broadening of the heavily stained rim as seen in some nuclei together with irregular heavy staining of the major part of others, also suggest a degradation of the nucleus (Fig. 13). However, the effects of tangential sectioning and varying degree of staining must be considered. Breakdown of the nucleus with loss of nuclear material was observed in some areas where the nuclei were situated in densely compressed fibrous tissues (Fig. 17).

One may speculate at which stage the cellular changes of the periodontal pressure zones become irreversible. So-called cloudy swelling, seen in the electron microscope as degranulation and swelling of the endoplasmic reticulum, is considered a regressive alteration which follows the impairment of the respiratory, energy-releasing mechanisms, that inhibit the cell in its continued excretion of sodium and water (*Robbins,* 1967). Also, mitochondrial swelling, which reflects influx of water and loss of electrolytes, is believed to be reversible (*Magee,* 1966). Mammalian liver can tolerate 30 to 45 min of total ischemia with complete recovery (*Baker,* 1956).

In the present study dilation of the endoplasmic reticulum and swelling of

mitochondria were observed after 30 min in the pressure foci. After 2 hrs similar changes were observed in the pressure region, while in the pressure foci rupture of cellular membrane was frequently seen.

These findings seem to be in accordance with *Baker's* (1956) conclusions. Accordingly, in rat molar periodontia, within a range of an orthodontic tipping force of 5—20 g, the critical period for the cells in the pressure foci seems to be from 30 min to 2 hrs. If cellular death is to be avoided, the active force must be discontinued within this period of time.

Cementoblasts, fibroblasts and osteoblasts showed no difference in cellular reaction to the orthodontic forces. This may seem surprising, since the enzymatic activity exhibited by the connective tissue cells of the periodontal ligament is not uniform, but is related to the location and function of the cells (*Fullmer*, 1967). According to *Macapanpan et al.* (1954) the osteoblasts disappeared before the cementoblasts in pressure zones in rats. The cells near the tooth surface seem to survive for a longer period than the cells near the alveolar wall in pressure zones in human material (*Reitan*, 1972). That no difference in the pattern of cellular degradation was observed in the present experiments may be due to too crude techniques. It is not considered possible to obtain reliable measurements of forces below 4 g with the appliance that was used. Furthermore, observations of cellular changes within the critical period of 30 min to 2 hrs should be included.

According to *Jande* (1971), the life of an osteocyte can be divided into three distinct phases. The cells show characteristic features of a formative, resorptive and degenerative period. In the present study the osteocytes near the hyalinized zones appeared to be in the formative and resorptive phases. These findings are not in agreement with *Oppenheim* (1944) and *Macapanpan et al.* (1954), who found degeneration of osteocytes with pyknosis of the nuclei and a noticeable number of empty lacunae in the alveolar bone near the areas of hyalinization. In the present study, osteocytes located within a few mμ from hyalinized zones, displayed no ultrastructural changes indicating degeneration or cell death after 5 days of orthodontic pressure. These observations do not agree with *Picton* (1969) who has described the bone near the hyalinized zone as necrotic.

On the basis of this and previous studies it must be concluded that »hyalinization» involves severe injury of fibroblasts and connective tissue cells, resulting in cell death. It appears that an initial phase of local necrosis in pressure zones is unavoidable with the range of forces presently used in orthodontic tooth movement. However, this necrosis seems to be limited to circumscribed areas in the periodontal ligament.

Acknowledgments. Financial support to this study was given by »L. Meltzers höyskolefond».

REFERENCES

Azuma, M., 1970: Study on histologic changes of periodontal membrane incident to experimental tooth movement. Bull. Tokyo med. dent. Univ. *17:* 149—178.

Baker, H. deC., 1956: Ischaemic necrosis in the rat liver. J. Path. Bact. *71:* 135—143.

Fullmer, H. M., 1967: Connective tissue components of the periodontium. In: *Miles, A. E. W.* (ed). Structural and chemical organization of teeth. Vol. 2. Academic Press, New York, p. 349.

Jande, S. S., 1971: Fine structural study of osteocytes and their surrounding bone matrix with respect of their age in young chicks. J. Ultrastruct. Res. *37:* 279—300.

King, D. W., Paulson, S. R., Hannaford, N. C. & A. T. Krebs, 1959a: Cell death. I. The effect of injury on the proteins and desoxyribonucleic acid of Ehrlich tumor cells. Amer. J. Path. *35:* 369—381.

King, D. W., Paulson, S. R., Hannaford, N. C. & A. T. Krebs, 1959b: Cell death. II. The effect of injury on the enzymatic protein of Ehrlich tumor cells. Amer. J. Path. *35:* 575—589.

Kronfeld, R. & J. P. Weinmann, 1940: Traumatic changes in the periodontal tissue of deciduous teeth. J. dent. Res. *19:* 441—454.

Kvam, E., 1967: Tissue changes incident to movement of rat molars. Thesis, Oslo.

Kvam, E., 1969: A study of cell-free zones following experimental tooth movement in the rat. Trans. Europ. orthodont. Soc. 419—435.

Macapanpan, L. C., Weinmann, M. D. & A. G. Brodie, 1954: Early tissue changes following tooth movement in rats. Angle Orthodont. *24:* 79—95.

Magee, P. N., 1966: Toxic liver necrosis. Lab. Invest. *15:* 111—131.

Moore, K. E. & T. M. Brody, 1960: Functional changes in liver mitochondria following in situ anoxia. Amer. J. Physiol. *198:* 677—681.

Oppenheim, A., 1944: Possibility for orthodontic physiologic movement. Amer. J. Orthodont. & Oral Surg. *30:* 277—345.

Picton, D. C. A., 1969: The effect of external forces on the periodontium. In: *Melcher, A. H. & W. H. Bowen* (eds.) Biology of the periodontium. Academic Press, London, New York. 363—419.

Reitan, K., 1951: The initial tissue reaction incident to orthodontic tooth movement as related to the influence of function. Acta odont. scand. Suppl. 6.

Reitan, K., 1969: Biochemical principles and reactions. In: *Graber, T. M.* (ed): Current orthodontic principles and techniques. W. B. Saunders. Philadelphia. Vol. 1: p. 56.

Reitan, K. & E. Kvam, 1971: Comparative behavior of human and animal tissue during experimental tooth movement. Angle Orthodont. *41:* 1—14.

Reitan, K., 1972: Personal communication.

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

Robbins, S. L., 1967: Cell injury and cell death. In: *Robbins, S. L.* (ed): *Pathology*. W. B. Saunders, Philadelphia and London.

Rygh, P., 1972: Ultrastructural vascular changes in pressure zones of the rat molar periodontium incident to orthodontic movement. Scand. J. dent. Res. *80:* 307—321.

Rygh, P. & K. A. Selvig, 1973: Erythrocytic crystallization in rat molar periodontium incident to tooth movement. Scand. J. dent. Res. *81:* In press.

Sandstedt, C., 1904: Einige Beiträge zur Theorie der Zahnregulierung. Nord. Tandl. Tidsskr. *5:* 236—256.

Sandstedt, C., 1905: Einige Beiträge zur Theorie der Zahnregulierung. Nord. Tandl. Tidsskr. *6:* 1—25.

Schwarz, A. M., 1932: Tissue changes incident to tooth movement. Int. J. Orthodont. *18:* 331—352.

Skillen, W. & K. Reitan, 1940: Tissue changes following rotation of teeth in the dog. Angle Orthodont. *10:* 140—147.

Smuckler, E. A., Iseri, O. A. & E. P. Benditt, 1962: An intracellular defect in protein synthesis induced by carbon tetrachloride. J. exp. Med., *116:* 55—71.

Stern, I. B., 1964: An electron microscopic study of cementum, Sharpey's fibers and periodontal ligament in the rat incisor. Amer. J. Anat. *115:* 377—410.

Trump, B. F., Goldblatt, P. J., & R. E. Stowell, 1965: Studies on necrosis of mouse liver in vito. Lab. Invest. *14:* 343—371.

Waldo, C. M. & J. M. Rothblatt, 1954: Histologic response to tooth movement in the laboratory rat. J. dent. Res. *33:* 481—486.

Address:
Research Laboratories,
School of Dentistry,
University of Bergen,
Årstadveien 17,
5000 Bergen, Norway