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BEHAVIOUR OF THE EPITHELIAL REMNANTS OF MALASSEZ FOLLOWING EXPERIMENTAL MOVEMENT OF RAT MOLARS

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An experimental study of incidence and prevalence (1), morphology (2) and proliferative activity (3) of the epithelial remnants of Malassez following orthodontic tooth movement of rat molars has been performed. It was observed that the experimental procedure influenced these 3 parameters of the marginal periodontal membrane. On the pressure side undergoing hyalinization, a marked reduction or a complete absence of epithelial remnants occurred.

On the tension side, increased prevalence of epithelial remnants occurred, compared to both the pressure side and to the control tooth. The epithelial remnants appeared to be transferred from a resting into a more proliferative state.

The present findings indicate that both pressure and tension produced by orthodontic tooth movement may influence the proliferative rate of the epithelial remnants.

Autoradiograms from animals injected with tritium labeled thymidine are useful for records of the proliferative cellular activity (*Amano, Messier & Leblond*, 1959). These include investigations on the cell renewal of the epithelial remnants of Malassez. Incorporation of labeled thymidine in these structures in untreated animals is well known (*Trowbridge & Shibata*, 1967; *Grupe, Ten Cate & Zander*, 1967; *Kvam & Gilhuus-Moe*, 1970), but it has also been suggested that the proliferative rate will be influenced by various stimuli (*Fischman & Greene*, 1964; *McHugh & Zander*, 1965; *Grupe, Ten Cate & Zander*, 1967). *Reitan* (1961) has shown that orthodontic tooth movement will influence the incidence and occurrence of these epithelial structures.

In the present investigation the morphology and proliferative activity of epithelial remnants located in the marginal portion of the periodontal membrane are recorded from rats injected with tritiated thymidine. The findings from the pressure and tension sides of experimentally moved rat molars are compared with observations of untreated, contralateral teeth.

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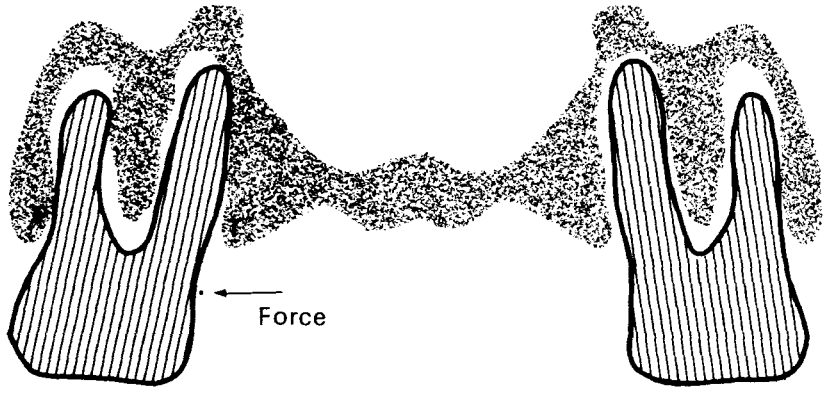


Fig. 1. Tracing showing the frontal section through maxilla and the first molars. Arrows indicate force direction

MATERIALS AND METHODS

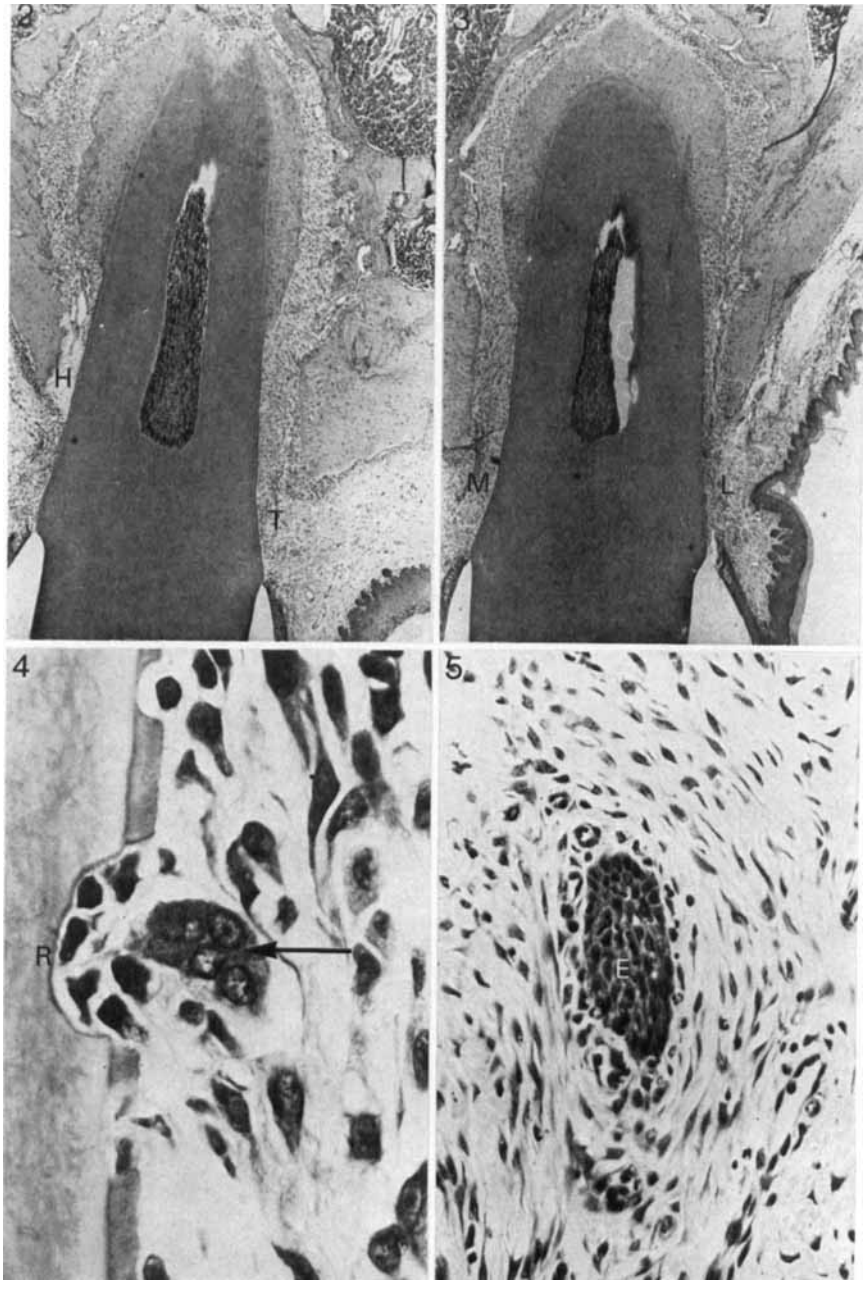
Materials. The present study is based upon two series of experimental animals comprising totally 38 young rats. The weight (in grams) of the animals in series I was 202 ± 20.4 at the start of the experiment, and 211 ± 25.8 at the end. In series II the corresponding weights were 242.5 ± 22.2, and 251.9 ± 21. The animals were kept two or three in plastic cages, and they received pellets and tap water *ad libitum* (*Koppang*, 1966).

Methods. In all animals an orthodontic appliance which was cemented to the upper incisor, excerted a continous force (about 20 grams) in a lateral direction for two days. The aim of this appliance, which is described in detail elsewhere (*Kvam*, 1967, 1970), was to create hyalinized zones on the pressure side of the periodontal membrane (Fig. 1).

The first series included 24 animals in which the orthodontic appliances were removed after 2 days. Three animals were then sacrificed consisting these observation periods:

1 hour, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days and 7 days after the removal of the orthodontic appliances. Sixty minutes prior to sacrifice each animal was injected with tritiated thymidine (H_3TDR*), 1 uCi/g body weight (*Tonna*, 1961).

Thymidine® Methyl (Methyl-T, Specific activity 5 Ci/mmol). The Radiochemical Centre, Amersham, England.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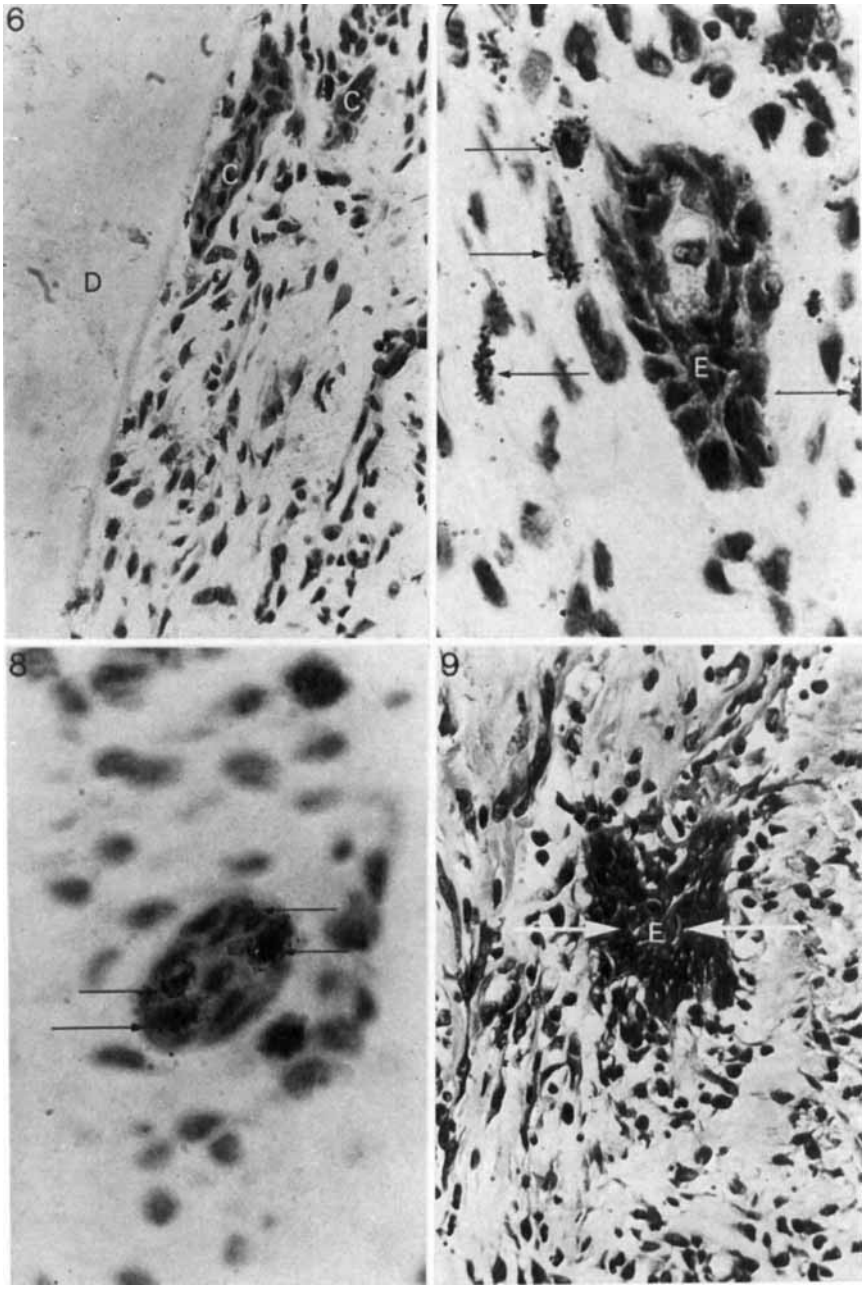
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In the second series of 14 animals the orthodontic appliances were removed after a force duration of 2 days. Nine animals were injected with H_3TDR (1 uCi/g body weight) at the removal of the appliance, and then sacrificed 12 hrs., 1, 2, 3, 4, 5, 6, 7, and 11 days after the injection. Five animals were injected three days after the removal of the appliance, and sacrificed 1, 2, 3, 4, and 5 days later. These series demonstrated the dilution of labeled epithelial cells following mitotic cell divisions. Each specimen was treated according to routine histologic and autoradiographic procedures, using the dipping method of autoradiography (*Joftes*, 1963). Each specimen was cut in a frontal plane securing that both 1. maxillary molars were cut at the same level of section.

In each animal the marginal portion of the periodontal membrane was examined at both the experimental and the contra-lateral (control) maxillary molar in areas shown in Figs. 1—3. In each animal the epithelial remnants of the periodontal membrane were estimated and the number of H_3TDR-labeled cells were recorded.

Fig. 2. Frontal section through experimental molar, 24 hours following removal of orthodontic appliances (First series). H: hyalinized zone on the pressure side. Orig. magn. × 12.5.

Fig. 3. Frontal section through control side (First series). Same specimen as fig. 1. M: Medial, marginal periodontal membrane. L: Lateral, marginal periodontal membrane. (Orig. magn. × 12.5).

Fig. 4. Resting epithelial remnants on tension side (arrow), 1 hour after removal of orthodontic appliances (First series). Note: resorptive defect of experimental tooth (R). (Orig. magn. × 80).

Fig. 5. Large epithelial remnants on experimental tension side (E). 5 days after removal of orthodontic appliances. (First series). (Orig. magn. × 80).

Fig. 6. Cord of epithelial cells (C) on medial, control side (First series). (Orig. magn. × 80).

Fig. 7. Autoradiograph. Nonlabeled, proliferating epithelial remnant (E) surrounded by labeled mesenchymal cells (arrows). Experimental, tension side, 3 days after removal of orthodontic appliances (Second series). (Orig. magn. × 330).

Fig. 8. Autoradiograph. Experimental tension side revealing an epithelial remnant with four H^3TDR labeled cells. 24 hours following removal of orthodontic appliances (First series). (Orig. magn. × 330).

Fig. 9. Proliferating epithelial remnant of experimental tension side, 1 hour after removal of orthodontic appliance (First series). Note: heavy proliferation and cytoplasmic, intercellular bridges (Arrows). (Orig. magn. × 130).

Table I.

Occurrence of epithelial remnants in the marginal portion of rat molar periodontal membrane

	EXPERIMENTAL SIDE			CONTROL SIDE	
OBSERVATION TIME	LATERAL (PRESSURE) SIDE	MEDIAL (TENSION) SIDE		MEDIAL SIDE	LATERAL SIDE
1 HOUR	□	□ ★ ★ ●		□	□
1 DAY		□ ★ □			
2 DAYS	□ □	□ ● □		□	□
4 DAYS				□	
5 DAYS		□ □ ★ □ □			□
6 DAYS		□ □ □ □			●

□ RESTING EPITHELIAL REMNANTS

● PROLIFERATING EPITHELIAL REMNANTS

★ H_3TDR LABELED EPITHELIAL REMNANTS

RESULTS

Generally, 2 main groups of epithelial remnants were observed in the marginal portion of the rat periodontal membrane. There was one small resting type present as small clusters (up to 10) of cells. In addition, a proliferating type was present characterized by more voluminous remnants, frequently forming cords of epithelial cells. The larger remnants were classified as proliferating.

In accordance with this classification the frequency and morphology of these epithelial remnants were recorded. The findings are shown in Tables I to III and Figs. 4—9.

First series. This series consisted of a total of 24 animals, 2 of these animals were rejected due to heavy round cell infiltration of the marginal periodontium. This reaction probably was a result of mechanical trauma produced by the orthodontic appliances.

Table II.

Occurrence of epithelial remnants in the marginal portion of rat molar periodontal membrane

	EXPERIMENTAL SIDE			CONTROL SIDE	
OBSERVATION TIME	LATERAL (PRESSURE) SIDE	MEDIAL (TENSION) SIDE		MEDIAL SIDE	LATERAL SIDE
12 HOURS	□				□ ●
1 DAY		□ □		□ □	□
2 DAYS				□	□
3 DAYS	□ □	● □ ★ □ □		□ □ □ □	□ □ □
4 DAYS	□ □ □	★ ● □ □ □		□ □ □	□
5 DAYS				□ □	□ □
6 DAYS				□	
7 DAYS					□

□ RESTING EPITHELIAL REMNANTS

● PROLIFERATING EPITHELIAL REMNANTS

★ H_3TDR LABELED EPITHELIAL REMNANTS

In the remaining 22 animals, 14 animals (64 %) revealed epithelial remnants at the experimental side, the control side or both in the marginal periodontal membrane. The distribution, morphology and classification of these remnants are presented in Table I and Table III.

Second series. In these series 14 animals were examined. Epithelial remnants were observed in 8 animals (58 %). The distribution, morphology and classification of these remnants are recorded in Table II and Table III.

On the *control* side in both series it was observed that the epithelial remnants were located closely to the surface or in the midportion of the periodontal membrane. The majority of remnants observed were of the resting type.

On the pressure side only few remnants were observed. Epithelial remnants were frequently observed on the tension side and many of these remnants were of the proliferating type.

Table III.

Occurrence of epithelial remnants in the marginal portion of rat molar periodontal membrane

	EXPERIMENTAL SIDE			CONTROL SIDE	
	LATERAL (PRESSURE) SIDE	MEDIAL (TENSION) SIDE		MEDIAL SIDE (SUM)	LATERAL SIDE
FIRST SERIES	3□	13□ 2● 4★		6□ 3●	
SECOND SERIES	6□	8□ 2● 2★		23□ 1●	
TOTAL	9□	21□ 4● 6★		29□ 4●	

□ RESTING EPITHELIAL REMNANTS
● PROLIFERATING EPITHELIAL REMNANTS
★ H_3TDR LABELED EPITHELIAL REMNANTS

H_3TDR labeled epithelial cells were observed on the experimental tension side. Of 6 H_3TDR labeled epithelial remnant observed, all were located on the experimental tension side.

In the first series, the most pronounced proliferative activities of the epithelial remnants were observed within the first 48 hours after the removal of the orthodontic appliances. In the second series the most prominent findings occurred at observations 1—4 days after the removal of orthodontic appliances.

DISCUSSION

The incidence and morphologic characteristics of the epithelial remnants in the rat molar periodontal membrane has been outlined by *Wentz, Weimann & Schour* (1950). These authors described 3 major groups of epithelial

remnants (i) a small, resting type, (ii) a proliferating type and (iii) a differentiating type. The present examination has been limited to 2 main groups (i) the small resting type and (ii) the proliferating type. The latter type was represented by more voluminous remnants, often forming cords of epithelial cells. The very large (differentiating) remnants in this study were also classified as proliferating.

The prevalence of epithelial remnants in the marginal portion of the periodontal membrane of the first maxillary molar in the rat in this study was 46 % (15/32) on the control side and 44 % (14/32) on the experimental side. These values correspond with the 47 % observed by *Wentz et al.* (1950) in the same portion of the rat periodontal membrane. The prevalence of epithelial remnants in this material as compared to that of *Wentz et al.* (1950), indicate that there do not appear to exist wide discrepancies in the number of epithelial remnants at the experimental and control sides. However, following orthodontic tooth movement the distribution of epithelial remnants demonstrates a wide variation between the lateral, hyalinized pressure side and on the medial, tension side.

Prominent differences in the incidence, prevalence, distribution and morphology of the epithelial rests of Malassez can be traced to these two portions of the periodontal membrane on the experimental side. It is assumed that these features may represent effects of the experimental tissue alterations following tooth movement.

A further discussion of these findings may therefore be focused towards the following factors:

Incidence and prevalence of epithelial remnants. It appears to exist a definite reduction in number of epithelial remnants within the hyalinized periodontal membrane following orthodontic tooth movement. Of 32 animals examined, only 5 (15 %) revealed apithelial remnants (of the small, resting type) on the pressure side. These results are corresponding with the observations of *Reitan* (1961). The low number of epithelial remnants on the pressure side is different from the high occurrence of remnants on the tension side, with an incidence of 43 % (14/32) in this area. Compared to the number observed on the control side, it appears that orthodontic tooth movement influence the function of periodontal epithelial remnants as observed on the control side, findings which are corresponding with earlier observations (*Wentz et al.* 1950, *Reitan* 1961).

Distribution and morphology of epithelial remnants. Wentz et al. (1950) demonstrated a high number of proliferating epithelial remnants in the supraalveolar portion of the periodontium, situated closely to the cementum or in the midportion of the periodontal membrane. In the present examination

the epithelial remnants of the control side was of the resting type, and only few were classified as proliferating.

On the experimental *tension* side the number of epithelial remnants of the proliferative type was high and more than 1/3 of those observed were of this type.

The fact that marked differences exist between the experimental pressure side and the tension side, and between the experimental tooth and the control tooth, indicate that both pressure and tension produced by orthodontic tooth movement may influence the proliferative rate of the epithelial remnants. In a population of young rats the majority of epithelial remnants of the periodontal membrane are nests of resting cells containing 5—15 cells (*Wentz et al.*, 1950; *Johansen*, 1970), evenly distributed in the marginal periodontium. The fact that proliferating epithelial remnants were observed in 10 of 3 remnants on the experimental tension side support the theories of an influence of mechanical traumas to epithelial cell proliferation in periodontal remnants (*Fischman & Greene*, 1964).

Incorporation of H_3TDR in epithelial remnants. In the present study 6 epithelial remnants of the marginal periodontium were recorded as H_3TDR labeled, all occurring on the experimental tension side.

The prevalence of H_3TDR labeled epithelial remnants of the tension side following orthodontic tooth movement in this study, may be a result of the experimental procedure and comparable with the increase in the number of cellular elements of the periodontal membrane (*Kvam*, 1970).

This increase in H_3TDR uptake, indicating an increased proliferative state, occurs rapidly — within 48 hours — after the cessation of the mechanical force. This is clearly vizualized in the first experimental series (flash-labeling) and the results are in harmony with those of *Ramfjord, Engler & Hiniker* (1966) and *Johansen* (1970).

The duration of this increased proliferation cannot be estimated from this experimental study, due to the fact that labeled epithelial remnants only was seen in one animal of the second series featuring the late labeling. However, the fact that the second series generally revealed a reduced uptake of H_3TDR, may indicate that a rapid reduction of proliferative activity in the epithelial remnant occur shortly after the cessation of the mechanical force. The withdrawel of the experimental forces appear to reverse this increased proliferation.

The mechanical effect of the experimental procedure on the epithelial remnants in this way appear to be parallel to the tissue reaction of the mesenchymal cells of the periodontal membrane (*Kvam*, 1967, 1970).

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