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# INCORPORATION OF TRITIATED THYMIDINE BY THE EPITHELIAL RESTS OF MALASSEZ AFTER ATTEMPTED EXTRACTION OF RAT MOLARS

*by*

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## INTRODUCTION

The epithelial rests of Malassez have attracted renewed attention in recent years and current techniques in histochemistry (*Ten Cate*, 1965; *Valderhaug & Nylen*, 1966), electron microscopy (*Valderhaug & Nylen*, 1966; *Nylen & Grupe*, 1969), autoradiography (*Diab & Stallard*, 1965; *Ramfjord et al*, 1966; *Trowbridge & Shibata*, 1967) and tissue culture (*Grupe et al*, 1967) have been applied to study the proliferative capacity of these cells. *Reeves and Wentz* (1962) separated the epithelial rests into groups as resting, proliferating and degenerating, but no mitotic figure indicating proliferation was found in their material. For further information about the occurrence in different species, the morphology and possible function of these epithelial cell rests in the periodontal membrane, the reader is referred to the extensive literature reviews of *Valderhaug* (1967) and *Grupe* (1968).

Mitotic figures (*Wentz et al*, 1950) as well as uptake of tritiated (3H) thymidine (*Trowbridge & Shibata*, 1967) have been reported under normal conditions in the rat. In explants of human periodontal membrane, epithelial rests were found to incorporate 3H thymidine in tissue cultures (*Grupe et al*, 1967).

A few reports give experimental evidence of the widely speculated capacity

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of the epithelial rests to proliferate. Proliferation of the epithelial rests in experimental conditions has been reported in the rat by *Fishman & Greene* (1964), but no mitotic figures were presented to substantiate their suggestion. *Ramfjord et al* (1966) reported uptake of 3H thymidine in epithelial rests five days after experimental gingivectomy in monkeys.

The purpose of this paper is to present further proof that the epithelial rests of Malassez have the capacity to proliferate following trauma as evidenced by the uptake of tritiated thymidine.

## MATERIAL AND METHODS

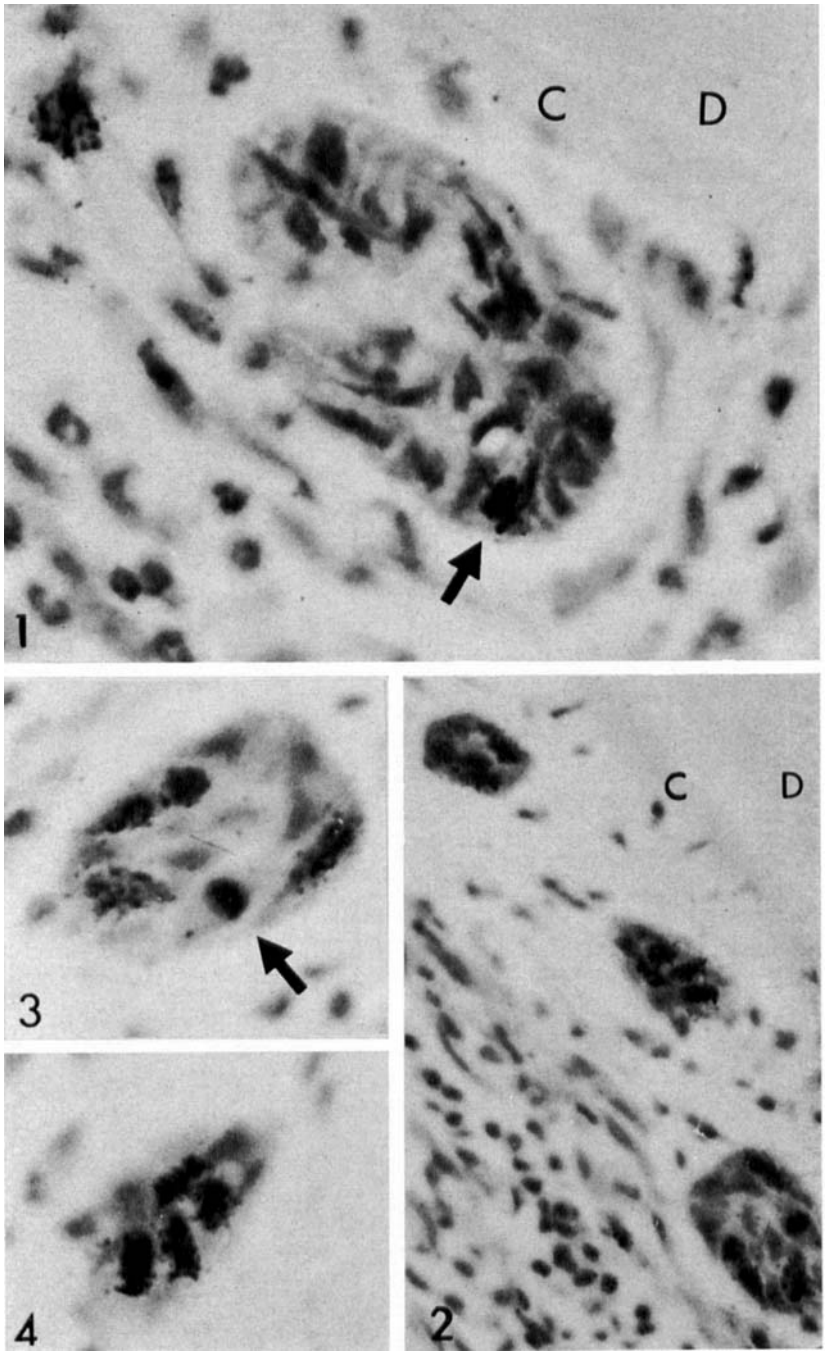
The following data were obtained after attempted extraction of the first mandibular molar in 18 male Wistar rats. In 4 rats the tooth fractured and the present report deals with changes of the epithelial rests of Malassez in the periodontal membrane of the roots that were left in the alveoli after the attempted extractions. Frontal sections of the heads were made in order to include the upper as well as the lower first molars. This plane of sectioning made it possible to evaluate the experimental and control sides in each section. Serial sections were made and prepared for autoradiographic and histologic examination in the routine manner. Details of the experimental design have been given elsewhere (*Johansen*, 1970).

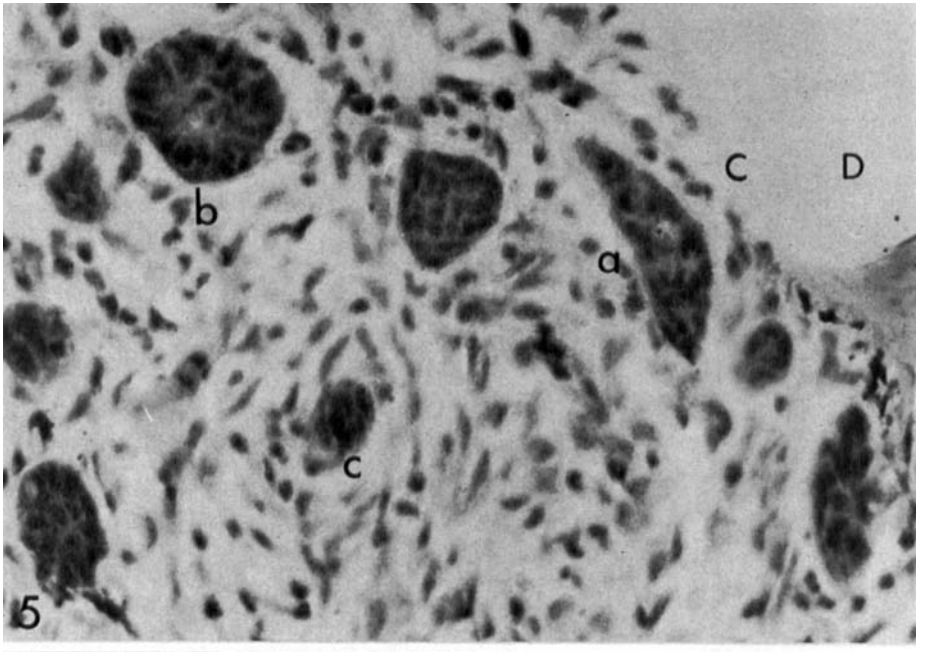
## OBSERVATIONS

*Control side.* In all 18 specimens of the maxillary and mandibular teeth epithelial rests were found on the control side. Their locations along the root surface and their distance from the bone and the cementum varied, but most often the epithelial rests were seen in the furcation and in the marginal third of the periodontal ligament of the first maxillary and mandibular molars and in close relationship to the cementum. Rests were located less frequently in the apical 2/3 of the periodontal ligament. The number of cells in each epithelial rest also showed great variation, from 5 up to 15 cells in the same section. The morphology of the rest was similar to that described by *Wentz* (1950), but no rests were of the »degenerating type».

*Experimental side.* Tooth fracture occurred in 4 of the animals which were sacrificed after 3—4—6 and 17 days observation time. Labelled epithelial rests were found in the furcation of the fractured tooth after 3 days (Fig. 1), after 4 days (Figs. 2, 3, 4) and after 6 days (Figs. 5, 6, 7). No labelling of the epithelial rests was seen in the 17 day specimen (Fig. 8).

PLATE I

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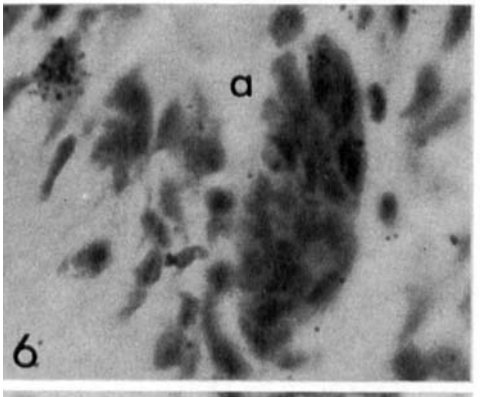
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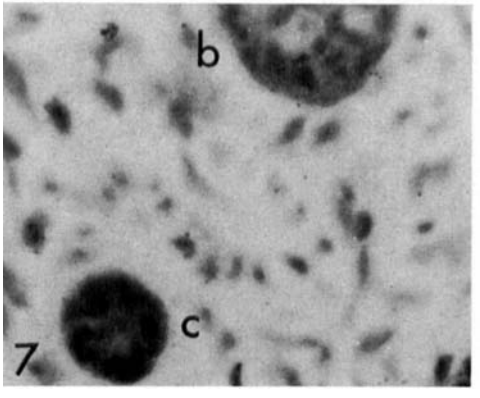
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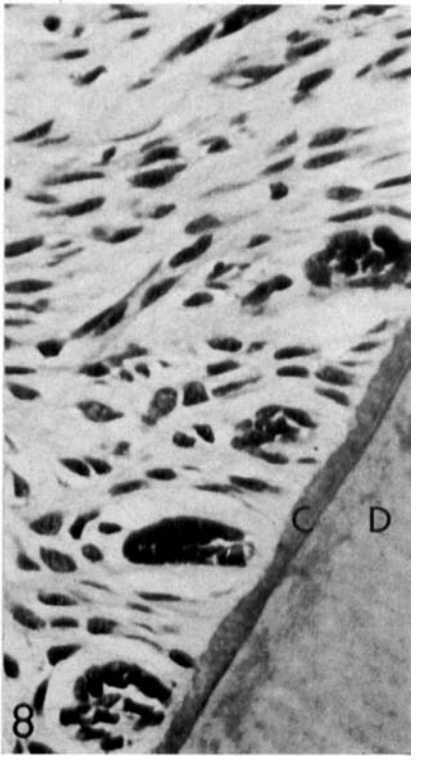
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## DISCUSSION

All specimens from the white male Wistar rats in this experiment showed the presence of epithelial rests of Malassez. This is surprising since the highest percentage ofrests in the study of Wistar rats of different ages was 71 % in a comparable age group in the study of *Wentz et al* (1950). It is possible that a strain difference exists, since the same authors reported a higher number in the gray Norway rat. On the other hand *Trowbridge & Shibata* (1967) found rests in only 2 out of 10 rats belonging to the Long-Evans strain. Whether or not these observations reveal real strain differences is difficult to ascertain. The frontal sections used in this study might offer some advantage in comparison to the conventional longitudinal sections because the sections show the periodontal membrane of 4 teeth in different parts of the specimen at the same time.

The epithelial rests of Malassez incorporated 3H thymidine in the 3—4 and 6 day specimens, but not in the 18 day specimen. The isotope was only available 1 hour prior to sacrifice of the animals. This suggests that the proliferative stimulus was reduced by 17 days after the trauma. *Ramfjord et al* (1966) found labelled rests 5 days after gingivectomy in monkeys using flash-labelling with 3H thymidine. They did not report labelled epithelial

Plate I

Fig. 1. One labelled nucleus (arrow) in an epithelial rest from the furcation area three days after attempted extraction. Dentin (D). Cementum (C). Autoradiograph, hematoxylin. Original magnification ×320.

Fig. 2. Three labelled epithelial rests from the furcation area four days after attempted extraction. Dentin (D). Cementum (C). Autoradiograph, hematoxylin. Original magnification ×320.

Fig. 3. The epithelial rest at the bottom of Figure 2 in a higher magnification. There are four labelled nuclei and one nonlabelled mitotic figure (arrow). Autoradiograph, hematoxylin. Original magnification ×320.

Fig. 4. The epithelial rest in the middle of Figure 2 in a higher magnification. There is intense labelling of four nuclei. Autoradiograph, hematoxylin. Original magnification ×320.

Plate II

Fig. 5. A cluster of epithelial rests in the furcation area. Six days after attempted extraction. Labelled nuclei are seen in some of the rests. Cementum (C). Dentine (D). Autoradiograph, hematoxylin. Original magnification ×200.

Fig. 6. A higher magnification of the rests labelled (a) in Figure 5. At least two nuclei are labelled. Autoradiograph, hematoxylin. Original magnification ×320.

Fig. 7. A higher magnification of two of the rests labelled (b & c) in Figure 5. One nucleus is labelled in (b) and two in (c). Autoradiograph, hematoxylin. Original magnification ×320

Fig. 8. Four epithealial rests in the furcation area seventeen days after attempted extraction Dentin (D), Cementum (C). Hematoxylin-eosin. Original magnification ×200.

rests at any other observation interval. The findings of labelled epithelial rests at 3—4 and 6 days intervals in this experiment might reflect the species difference in the prevalence of rests rather than differences in stimuli for proliferation.

In this study it proved difficult to establish that the epithelial rests proliferated using morphological criteria alone. In the 17 day specimen the finding of 3 epithelial rests close to each other would suggest that proliferation had occurred, but the autoradiograph revealed no labelled cells. If the tritiated thymidine had been available longer than 1 hour, labelling might have been found.

The frontal sectioning used in this material was well suited to exclude any possibility of a connection between the epithelial rests of Malassez and the gingival epithelium. Downgrowth of the pocket epithelium into the furcation areas has been reported in old rats by *Wentz et al* (1950). This might easily be misinterpreted as proliferating epithelial rests, if longitudinal sectioning of the specimen had been used (*Fishman & Greene*, 1964). The use of tritiated thymidine has almost eliminated previous speculations about cell proliferation, when no mitotic figures could be seen, but careful serial sectioning is still important to discard the possibility of a connection with the pocket epithelium.

The design of this experiment did not allow for any definite conclusion as to the nature of stimulus responsible for the reported uptake of the 3H thymidine in the epithelial rests. No uptake was found at the control sites. The labelled rests were all located in the vicinity of inflammatory reactions around the remaining root. In an earlier report (*Johansen*, 1970), connective tissue cells of the periodontal membrane distant to the site of inflammation were also seen to proliferate. Therefore, it seems possible that mechanical and inflammatory stimuli may influence the proliferative capacity.

The possible role of the epithelial rests of Malassez in pocket formation (*Orban & Weinmann*, 1942; *Grant & Bernick*, 1969) and in the development of radicular cysts (*Hill*, 1930) has been widely discussed. The observations in this study show that epithelial rests have the capacity to proliferate when stimulated. It is, therefore, conceivable that they may take part in the formation of the epithelium of the pocket walls in periodontitis and in the epithelial lining of radicular cysts.

## SUMMARY

Epithelial rests of Malassez were observed in the periodontal membrane of the first molars in both the upper and lower jaws in all the Wistar rats used

in this study. No uptake of tritiated thymidine was found in these rests at the control sites.

Following tooth fractures resulting from unsuccessful extraction attempts, labelled epithelial rests were observed on the 3rd, 4th and 6th day, but not on the 17th day. This indicates that trauma and subsequent inflammation are stimuli for proliferation of these cells.

RÉSUMÉ

INCORPORATION DE THYMIDINE TRITIÉE DANS LES DÉBRIS ÉPITHÉLIAUX DE MALASSEZ APRÈS TENTATIVE D'EXTRACTION DES MOLAIRES CHEZ LE RAT

Des débris épithéliaux de Malassez ont été observés dans le desmodonte des premières molaires des deux mâchoires chez tous les rats Wistar utilisés pour cette étude. Aucune absorption de thymidine tritiée n'a été trouvée dans ces débris au niveau des zones témoins.

Après des fractures dentaires résultant de tentatives non réussies d'extraction, des débris épithéliaux marqués ont été observés le 3ème, le 4ème et le 6ème jour, mais pas le 17ème jour. Ce fait indique que le traumatisme et l'inflammation en résultant stimulent la prolifération de ces cellules.

ZUSAMMENFASSUNG

AUFNAHME TRITIERTEN THYMIDINS VON MALASSEZISCHEN EPITHELRESTEN NACH VERSUCHTER EXTRACTION VON RATTENMOLARZÄHNEN

Malassezische Epithelreste wurden in der periodontalen Membrane des ersten Molarzahnes in sowohl Ober- als auch Unterkiefer in allen Wistar-Ratten, die in dieser Untersuchung benutzt wurden, beobachtet. Eine Aufnahme von tritiertem Thymidin wurde in diesen Resten der Kontrollstellen nicht gefunden.

Nach Zahnfrakturen, verursacht durch erfolglose Extraktionsversuche wurden radioaktive Epithelreste am 3., 4. und 6. Tag beobachtet, aber nicht am 17. Tag. Dies zeigt dass Trauma und darauffolgende Entzündung Stimuli für die Wucherung dieser Zellen sind.

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