Zeitschrift: Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften = Bulletin de l'Académie suisse des sciences médicales = Bollettino dell' Accademia svizzera delle scienze mediche

Herausgeber: Schweizerische Akademie der Medizinischen Wissenschaften

Band: 11 (1955)

Heft: 4-5

Artikel: Daily variations of mitotic rate and inflammatory cell migration in the epithelium of the intermolar rat papilla

Autor: Mühlemann, H.R. / Hartl, S.

DOI: https://doi.org/10.5169/seals-307232

C. D. 616.31:612.014.2

From the Department of Operative Dentistry and Periodontology
Dental Institute, University of Zurich, Switzerland

Daily Variations of Mitotic Rate and Inflammatory Cell Migration in the Epithelium of the Intermolar Rat Papilla

By **H. R. Mühlemann** and **St. Hartl**

In a previous report significant daily variations in the mitotic activity of the periodontal membrane and the retromolar epithelium in the rat were shown (1). A positively correlated morning high and a night low in cell division were also recorded for the epidermis of the ear lobe and for eosinophil counts in tail blood (2). However a significant day-night-difference in number of mitoses was not detected in the epithelium of the *whole* interdental papillae ($P_{\chi^2} = .32$). It was assumed (1) that intraepithelial inflammatory cell infiltration could be one of the factors responsible for the lack of the 24-hour mitotic rhythm of the whole papilla. This was further suggested by the observation of a higher proportion of mitoses in non-infiltrated than in infiltrated areas of the papillary epithelium when day and night samples were pooled.

It is the purpose of this investigation to present the results a) of cell division rate within the infiltrated and the non-infiltrated epithelium of the rat intermolar papillae and b) of daily variations in intraepithelial inflammatory cell infiltration. Details about the distribution of mitoses in the papillary epithelium (3), and the concentration and distribution (4) of round cell infiltration within the same structures were reported elsewhere.

Material and methods

Thirty male black rats were used for this study. They were 5 months old at the time of investigation and showed no signs of disease. From weaning and throughout investigation, Purina Fox Chow and tap water were available to the rats ad libitum. For three weeks prior to the start of the study, the animals were maintained in single cages. They were kept in a room maintained at a temperature of 25° ± 1° C., and illuminated by artificial light only. The lights were turned on at 6:00 a.m. and off at 6:00 p.m. by means of an automatic switch. Feeding of the rats

and the cleaning of the cages were limited to a certain time of day (around 5:30 p.m.), and did not extend over a period longer than thirty minutes. The rats were divided into two groups: Group I, comprising 15 animals, weighing 280 ± 11 grams, was sacrified between 9:21 and 11:56 p.m. ("*night* rats"), Group II, 15 animals, weighing 281 ± 7 grams, was sacrified between 6:35 and 8:48 a.m. ("*day* rats"). The rats were killed by decapitation at these two times of day. After decapitation the jaws were immediately dissected and fixed in Zenker-formalin solution within 2 minutes. After decalcification with 5% nitric acid, followed by celloidin embedding, histological sections were prepared from all the lower jaws.

The lower left jaw was sectioned in a mesiodistal direction. The sections were stained with haematoxylin and eosin. Enlarged photographic prints were made from the 2 interdental papillae between the three molars. On these prints the epithelial areas free of any migrating round cells (= non-infiltrated areas) were demarcated from the infiltrated areas. Mitoses and non dividing cells were counted in the infiltrated and non-infiltrated areas of 102 sections and recorded on the photographic prints. The number of migrating inflammatory cells in the infiltrated areas was also recorded.

Findings

The findings of the mitotic activity and degree of round cell infiltration in day and night rats are assembled in table 1. There was a *significant day-night-variation in mitotic activity in non-infiltrated areas, but not in the infiltrated areas.* Mitotic rate in regions free of inflammatory cells was higher in day rats. Both, day- and night rats, had higher mitotic indeces in the non-infiltrated epithelial zones.

The *number of inflammatory cells migrating* through the papillary epithelium was also *higher in the day rat sample.* (67.1 ± 5.7 round cells per 100 epithelial cells), the difference with the night rat sample (48.0 ± 3.2) being at a significance of the 7‰ level. The *number of epithelial cells* counted in the *non-infiltrated zones* was 8213 for day rats, 16 614 for night rats. Fig. 1 and 2 illustrate the demarcation zone of an infiltrated and non-infiltrated epithelial zone. Infiltration was more pronounced in the epithelial attachment (EA) than in the tip of the papillae (OE).

Discussion

The separate counts of mitoses in non-infiltrated an infiltrated areas of the papillary epithelium allowed the detection of a *day-night-mitosis-rhythm* in the regions free of round cell migration as it was previously

Table 1

Mitotic and Round-cell-infiltration Indices in Non-Infiltrated and Infiltrated Areas of the Epithelium of the Interdental Papilla of "Day-" and "Night" Rats

	Non-Infiltrated Areas					
	No. Rats	No. Mitoses	No. Round Cells	No. Epithelial Cells	Mitotic Index [1]	Significance P
Day rats	15	327	0	8 213	3.981 ± 0.2157	<.001
Night rats	15	443	0	16 614	2.666 ± 0.1248	

	Infiltrated Areas								
	No. Rats	No. Mitoses	No. Round Cells	No. Epithelial Cells	Mitotic Index [1]	Significance of Mitotic Indices P_t		Infiltration Index [2]	Significance P_t
Day rats	15	598	20 444	30 339	1.972 ± 0.0798	.24	<.001 [3]	67.1 ± 5.7	<.007
Night rats	15	666	12 614	31 634	2.105 ± 0.0807		<.001 [4]	48.0 ± 3.2	

[1] Average number of mitoses per 100 cells, ± standard error.

[2] Average number of round cells per 100 epithelial cells, ± standard error.

[3] Significance of difference in mitotic activity of day rats in infiltrated and non-infiltrated areas.

[4] Significance of difference in mitotic activity of night rats in infiltrated and non-infiltrated areas.

found (1) in the *non-infiltrated retromolar epithelium* of the same rats. However the level of the mitotic activity appears to be lower in the retromolar epithelium. In table 2 the findings in the retromolar epithelium free of any inflammation and in the non-infiltrated papillary epithelial areas are compared[1]. The difference in the mitotic rate between both regions suggests the conclusion that *subepithelial inflammatory phenomena adjacent to the non-infiltrated areas of the intermolar papillae stimulate cell division both in day and night rats.*

Table 2

Mitotic Activity in the Retromolar and Non-Infiltrated Papillary Epithelium of the Rat

Time of Sampling	Average Number of Mitoses		
	In Retromolar Epithelium		In Non-Infiltrated Areas of Papillary Epithelium per 100 Cells
	per 2000 Cells (original report)[1]	Calculated per 100 Cells	
Day	14.0	0.7	3.98
Night	3.6	0.18	2.66

[1] The original counts of mitoses in the retromolar region were related per 2000 cells and they were performed by an other investigator (H. R. M.) than those for the mitoses in the papillae (St. H.).

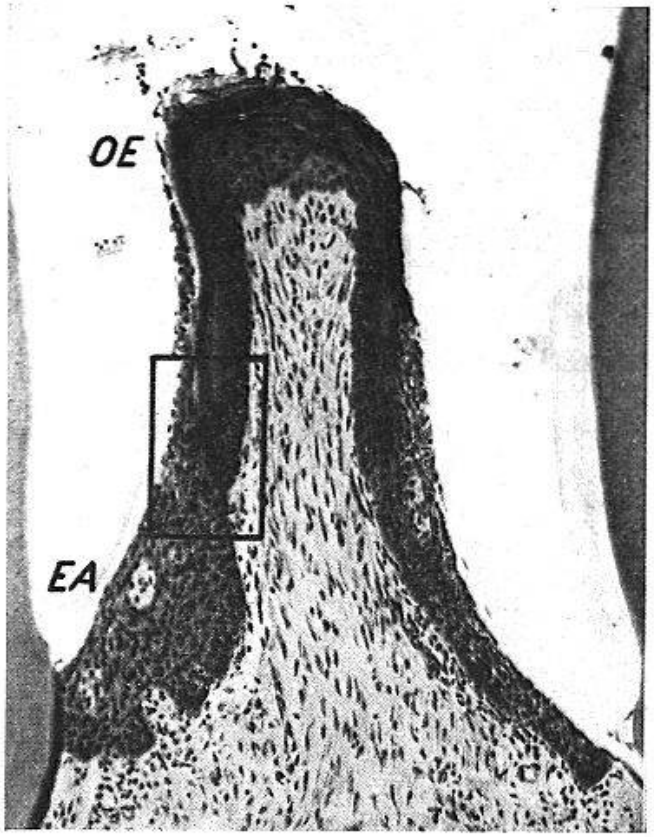

Fig. 1. Interdental papilla of the rat molar.
EA: Infiltrated epithelial area.
OE: Non-infiltrated epithelial area.

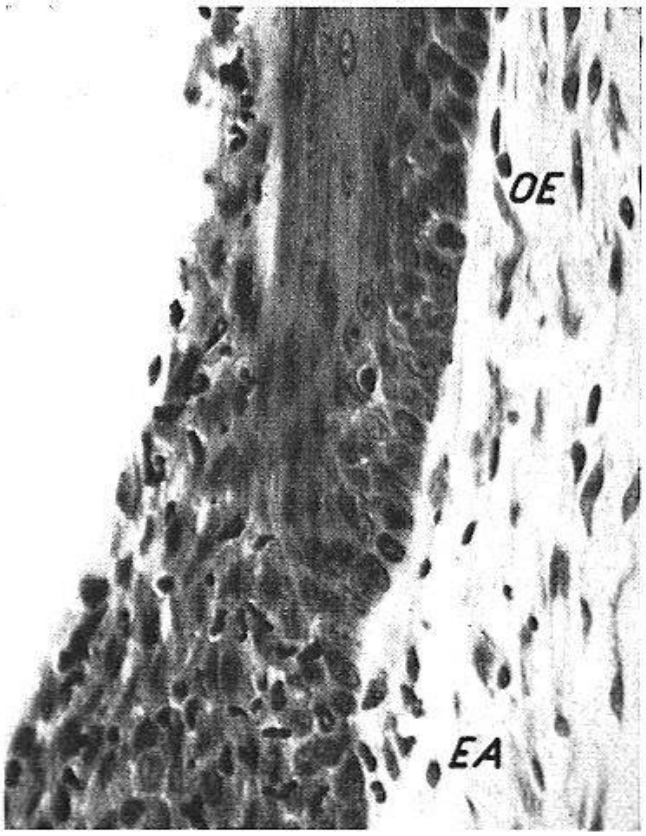

Fig. 2. Enlargement of fig. 1. Demarcation zone of infiltrated (EA) and non-infiltrated epithelial areas (OE).

Active cell division, but *no 24-hour-mitosis-periodicity* was recorded for the *infiltrated areas* which always included the epithelium of the epithelial attachment. Pooling of the data of mitoses in infiltrated areas and non-infiltrated regions gives no significant day and night difference in mitotic rate, confirming the observation already reported (1).

The annihilation of the 24-hour-mitoses rhythm seems to be due to an interference with the factors that are responsible for the *day-time high* in cell division (3.98 mitoses per 100 cells in *non-infiltrated areas* of *day rats* and 1.9 mitoses per 100 cells in *infiltrated areas* in *day rats* [table 1]). It is evident that round cell infiltration may be one of the interfering causes: Inflammatory cell infiltration within the epithelium was definitely more pronounced in day rats than in night rats. Both the *extension* and the *concentration* of *inflammatory cell infiltration* were significantly greater in day rats. The infiltrated surface (expressed by the number of non dividing epithelial cells) was twice as small in day rats. Despite the distributions of round cells over a greater epithelial area in the day rat sample, the *concentration* of infiltration (infiltration index) was also more pronounced.

The *infiltration increment* in the day samples is assumed to be functionally related with the lack of day-time *increment of cell division*, suggesting therefore an *inhibition of mitosis*. This statement is in contrast with the statement in the first part of this discussion. However the discrepancy of conclusions may be only relative and could be explained by taking into consideration the degree or the severity of inflammation. The interdental papilla has to be considered as one functional unit. Inflammatory phenomena that are more pronounced at the base of the conical papilla, probably also influence the tip of the papilla. Evidence was given that the epithelial mitotic rate of the papilla as a whole was stimulated. We suppose that inflammatory processes in the subepithelial connective tissue at the base of the papillae are the cause of it. (Higher mitotic activity of both infiltrated and non-infiltrated regions when compared to the non-inflamed retromolar area.) The annihilation of the mitotic periodicity in the infiltrated apical regions of papillary epithelium is the consequence of a relative inhibition of mitosis by the day-time increment in inflammatory cell infiltration. Despite this inhibitation the absolute mitotic rate is still somewhat greater than in zones completely free of inflammation. These findings suggest a negative correlation between degree of infiltration and mitotic rate. Correlation coefficients computed for these variables however were not significantly different from zero. The small size of the samples may be one reason for it. On the other hand, it might be assumed that lack of significant cor-

relation could be the result of stimulation of cell division by discrete infiltration and inhibition by more pronounced infiltration.

Summary

Thirty black male rats, 5 months of age, were studied under standardized circumstances. A marked mitotic 24-hour-periodicity was noted for the non-infiltrated areas of the epithelium of the interdental papillae. The diurnal rhythm of epithelial cell division was absent in the infiltrated areas of the papilla. Intraepithelial inflammatory cell infiltration also showed a significant diurnal low and high, with peaks identical when compared to the daily variations in mitotic activity of epithelium free of inflammatory cells. The relationship between inflammation and mitotic rate in the structures studied is discussed.

The authors wish to express their appreciations to Dr. H. A. Zander for the permission to use in this paper material prepared at the Department of Periodontology at the University of Minnesota.

Portion of this work was done with the aid of a grant from the "Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung".

Zusammenfassung

Von 30 schwarzen männlichen, 5 Monate alten, unter standardisierten Bedingungen aufgezogenen Ratten wurde die eine Hälfte am Morgen (Tagratten) und die andere Hälfte am Abend (Nachtratten) dekapitiert. In sagittalen Unterkieferschnitten wurde die Zahl der Mitosen und diejenige der wandernden Entzündungszellen im Epithel der interdentalen Molarenpapillen gezählt. In *nicht-infiltrierten Zonen* des Papillenepithels wurde eine signifikante Tag-Nacht-Periodizität der mitotischen Aktivität gefunden. Der *Mitosenrhythmus fehlte* in den mit Rundzellen *infiltrierten Epithelabschnitten*. Hingegen ließ sich in letzteren Zonen eine signifikante Tag-Nacht-Periodizität des Infiltrationsgrades feststellen, wobei der stärkere Infiltrationsgrad der Tagratten mit der höheren mitotischen Aktivität (Tagratten) zusammenfiel. Die Beziehungen zwischen entzündlicher Rundzelleninfiltration und mitotischer Aktivität werden diskutiert.

Résumé

De 30 rats noirs mâles, âgés de 5 mois, élevés dans des conditions standardisées, une moitié fut décapitée le matin (rats de jour) et l'autre moitié le soir (rats de nuit). Sur des coupes histologiques sagittales du maxillaire inférieur on dénombra les mitoses et leucocytes en migration dans l'épithélium des papilles interdentaires des molaires. Dans les *zones épithéliales non infiltrées*, on trouva une nette *périodicité jour-nuit* dans

l'activité mitotique. Le *rythme* mitotique *manquait* dans les *secteurs épithéliaux infiltrés* de leucocytes. Par contre, on put noter, dans les derniers, une nette *périodicité jour-nuit du degré d'infiltration leucocytaire*. Le degré d'infiltration le plus élevé des rats de jour coïncidait avec l'activité mitotique la plus forte (rats de jour). Les rapports entre l'infiltration inflammatoire et l'activité mitotique sont discutés.

Riassunto

Vennero allevati in condizioni standard 30 ratti maschi, fino all'età di cinque mesi.

Metà di questi furono sacrificati al mattino, l'altra metà di sera. Vennero quindi preparate sezioni istologiche sagittali del mascellare inferiore, ed in particolare furono contate le cellule in mitosi presenti e quelle facenti parte dell'infiltrato flogistico. Caratteristico è il fatto che le mitosi si riscontrano in copia maggiore in quei ratti sacrificati al mattino. Questa periodicità delle mitosi non venne peraltro riscontrata in quelle zone epiteliali presentanti infiltrati flogistici, zone peraltro in cui una periodicità analoga sussiste per quel che riguarda il numero delle cellule di infiltrazione flogistica. I rapporti intercorrenti tra l'attività mitotica ed il grado di infiltrazione vengono trattati.

1. *Mühlemann, H. R., Zander, H. A.*, and *Halberg, F.:* J. dent. Res. **33,** 459 (1954). – 2. *Zander, H. A., Houghlum, M. W., Halberg, F.*, and *Mühlemann, H. R.:* Amer. J. Physiol. **177,** 361 (1954). – 3. *Hirt, Ch. M., Hartl, St.*, and *Mühlemann, H. R.:* The Distribution of Mitoses in the Epithelium of the Interdental Papillae of the Rat Molar. J. Periodontol. **26,** 229 (1955). – 4. *Fehr, C.*, and *Mühlemann, H. R.:* Parodontologie **8,** 159 (1954). – 5. *Hartl, St.:* Die 24-Stunden-Periodik der mitotischen Aktivität und der Rundzelleninfiltration im Epithel der Interdentalpapilla der Ratte. Diss. Zürich 1955 (in press).