PHYSIOLOGICAL ASPECTS OF THE GINGIVAL POCKET

AN EXPERIMENTAL STUDY

by

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The present paper deals with two aspects of the physiology of the gingival pocket,

(1) the turn-over of the epithelial cells, and

(2) the presence of leukocytes in the gingival tissue fluid.

Recently it was demonstrated that there is an outward seepage of fluid from clinically normal gingival pockets (*Brill & Krasse* 1958, *Brill & Björn* 1959). Moreover, mitotic activity is likely to occur in the epithelial lining of the normal gingival pocket*) approximately at the same rate as in other oral epithelia (*Hirt, Hartl & Mühlemann* 1955, *Greulich* 1961, *McHugh* 1961). This would imply that the surface cells are desquamated at the same rate, although this has not yet been substantiated. If cells are shed from the crevicular epithelium, they would most likely be found in this gingival fluid.

The purpose of the present experiments was to establish a retention of any cellular elements originating from the crevicular tissues, thereby making a histologic examination of the cellular content of the gingival fluid feasible.

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*) In this article no distinction is made between "pocket" and "crevice", as such a distinction is poorly defined.

MATERIAL AND METHOD

Three healthy dogs were used. Prior to the experiment, the gingivae were inspected and their clinical characteristics assessed. Under Nembutal anesthesia forty-nine clinically normal gingival pockets were sealed with an alcoholic solution of colophony which was painted on the marginal parts of the gingivae and the crowns of the teeth. In the experiments lasting less than 6 hours, during which the animals were anesthetized, the coating was made quite thick, and no other means were used to seal the pockets. In the experiments lasting 12 hours or more — during which time the dogs were partly awake — additional precautions were taken in order to avoid a breaking of the seal during function. On top of the layer of colophony was placed an acrylic crown which completely covered the gingival margin. The crown was filled with surgical cement and locked with amalgam (Fig. 1).

The observation period varied from 1 to 48 hours. After fixation in 10 % neutral formalin, the specimens were decalcified in 5.2 % nitric acid. During the histologic procedure efforts were made to keep the marginal cover in place as long as possible. The acrylic crowns were finally dissolved in chloroform before the specimens were embedded in paraffin. The sections were cut at five microns and stained in hematoxylin and eosin.

FINDINGS

The microscopic examination showed in all cases non-keratinized epithelial cells making up a continuous lining of the gingival pocket ending at the cemento-enamel junction (Figs. 2, 3, 4, and 5). Thickening of the epithelium was not seen in the deeper parts of the pockets, although a few and inconspicuous rete pegs were present in the marginal area (Figs. 2, 3, and 4). Similar conditions were observed when neighboring untreated teeth were examined. Usually the width of the epithelial cuff*) ranged from

*) "Epithelial cuff" is in this article used both when the epithelial cells were found in contact with the enamel and when accumulations of leukocytes had caused a separation of these two tissues.

8 to 15 layers of cells. Mitoses were often seen in the basal layers all along the epithelial cuff (Figs. 2A and 4C).

In the *short term experiments* (1—6 hours), neutrophilic leukocytes were consistently seen within the normal epithelial cuff, presumably on their way towards the surface (Figs. 2 B and C). In these cases the adjacent connective tissue revealed no abnormal aggregations of leukocytes or other signs of inflammatory reactions (Figs. 2B and 3C).

In the *long term experiments* (12—48 hours), the appearance of the epithelial cuff did not basically deviate from the description already given (Figs. 4 and 5), except for the fact that leukocytes were more frequently found in the epithelial layer. Accumulations of the same type of cells in the subepithelial connective tissue were consistent findings, although the extention of the infiltration varied. In no instances were neutrophiles seen in the keratinized epithelium facing the oral cavity.

The method employed in this series allowed a close examination of the cellular elements emanating from the crevicular tissues. In all cases smaller or larger concentrations of cells could be seen within the gingival pocket. In the teeth with short periods of observation (1—6 hours), the cells were clustered near the gingival margin (Figs. 2 and 3). The formed elements of the trapped material were exclusively neutrophilic leukocytes and epithelial cells, separated from each other by an amorphous intercellular material, in which empty spaces were regularly observed (Figs. 2A and 3A). In the long term experiments (12—48 hours), the gingival pockets were filled with cells to the extent that the free gingiva was forced away from the enamel surface (Figs. 4 and 5). These specimens also showed desquamated epithelial cells and neutrophiles in varying stages of degeneration (Figs. 4 F, G, H). This pertained particularly to the cells adhering to the organic remnants of the enamel (Fig. 5E) and in the area of the gingival margin.

DISCUSSION

This experimental investigation has shown that when clinically normal gingival pockets are sealed at the margin, cellular elements dispersed in an intercellular material are retained between the

epithelial cuff and the enamel surface. The cellular fraction comprises desquamated epithelial cells and neutrophilic leukocytes. Most likely the amorphous substance is the remnants of the gingival tissue fluid.

The gradual increase in the amount of trapped material tends to show that desquamation of epithelial cells from crevicular epithelium, the migration of neutrophiles and the flow of tissue fluid through the same epithelium are continuously occurring phenomena.

The solution of colophony did not seem to exert any irritating effect on the gingiva as no difference as to the appearance could be found when treated and untreated specimens were examined. The possibility that the artificial retention of fluid and cells could enhance the processes involved, does not obscure the fact that an initial transudation has been present.

The present findings seem, therefore, to corroborate the observations made by *Brill* and co-workers, that there is a continuous transudation of tissue fluid into the clinically normal gingival pockets. As yet, no studies have been made on the mechanism of this process. There are reasons to believe that this fluid, as tissue fluid in general, originates from the blood (*Brill & Krasse* 1958) and passes from the subepithelial connective tissue between or through the epithelial cells of the cuff. There is also some basis for the assumption that the gingival tissue fluid is of the same composition as blood plasma (*Brill & Brönnestam* 1960). The possibility that the fluid is a product of secretion must be discarded as this area contains no glands. The explanation of a continuous passage of fluid through the crevicular epithelium may be found in the slightly higher hydrostatic pressure of the tissue fluid (approx. 10 mm Hg, *Evans* 1952) as compared to atmospheric pressure. The absence of keratinized cells in the epithelial cuff facilitating such a process must also be considered.

With regard to the neutrophilic leukocytes, no counts were made, but it seems as if they are more numerous than the epithelial cells. The presence of neutrophiles within the crevicular epithelium, indicates that these cells normally migrate from the subepithelial connective tissue into the gingival pocket. The findings of the same cells in normal epithelium covering a non-inflammatory connective tissue are not in agreement with the statement of *Lundquist* (1940). He

maintained that discontinuity of the epithelial lining is a prerequisite for the migration of leukocytes from subepithelial tissue into the gingival pocket. On the other hand, the present findings are in agreement with the observations made of neutrophilic leukocytes arraying through the epithelium, while few were seen in the adjacent *lamina propria* (*Grant & Orban* 1960).

Brill & Krasse (1958) did not indicate the part of the gingival pocket where the passage of the fluid took place. The present observations of leukocytes from the gingival margin to the cemento-enamel junction tend to show that the entire length of the epithelial cuff is involved, and that the localization of the escaped cells at the gingival margin, demonstrated in the short term experiments, is due to the outward flow of the gingival fluid.

The morphologic characteristics of the leukocytes in the gingival space suggest that the majority of them are vital for some time after having passed through the epithelium. Whether they are capable of exercising phagocytic activity, and in that case how long time this activity will last must be elucidated by further investigations.

The occurrence of mitotic figures in the basal layer along the entire length of the epithelial cuff is in accordance with earlier observations (*Hirt & al.* 1955, *Greulich* 1961, *McHugh* 1961). This, together with the finding of desquamated epithelial cells in the gingival fluid, gives the evidence of a continuous renewal of the epithelial cuff.

With the method employed in the present experiments it was impossible to assess the rate of renewal of the epithelial cuff. However, in these teeth the width of the epithelial layers was doubled in the course of 24 hours (Fig. 5). Irrespective of the turn-over rate, the continuous renewal of the epithelium implies that an organic union between the surface cells and the enamel would have to be renewed constantly.

Gottlieb (1921) introduced the term "der Epithelansatz" — the epithelial attachment — to characterize the relationship between the gingival epithelium and the enamel surface. The concept that the gingival epithelium is, in the true sense of the word, attached to the enamel surface is still widely accepted (*Orban & al.* 1956, *Weinreb* 1960). On the other hand, experimental investigations

have shown that the supposed union is very weak (*Waerhaug* 1952, 1960, *Zander* 1956). On the basis of findings like these and series of other experimental investigations (*Waerhaug* 1956, 1957, *Waerhaug & Löe* 1957, 1958), the term epithelial cuff was suggested to connote that the connection between the gingival epithelium and the enamel or cementum depends on adhesion. The present results and recent works on the development (*McHugh* 1961) and the physiology (*Brill & al.* 1958, 1959, 1960) of the gingival pocket tend to support this view.

Orban and co-workers (1955) emphasized that an epithelial attachment is an important link in the continuous cover of the body surface, protecting the underlying tissues against chemical damage or bacterial irritation. However, the outward flow of fluid from normal gingival pockets and the indications of the presence of antibodies (*Brill & Brönnestam* 1960) and phagocyting cells in this fluid appear to constitute an essential mechanical and antibacterial defence mechanism against local irritants.

SUMMARY

In order to elucidate some physiological aspects of the gingival pocket, a series of animal experiments was carried out. In three dogs 49 clinically normal gingival pockets were sealed at margin for periods ranging from 1 to 48 hours.

The histologic examination of the specimens revealed that the pockets contained desquamated epithelial cells and neutrophilic leukocytes dispersed in an amorphous substance. The amount of trapped material increased with increasing observation time.

The findings of mitotic figures along the entire length of the epithelial lining of the pocket and the desquamation of the surface cells support the view that the epithelial cuff is constantly renewed.

The presence of neutrophilic leukocytes within the epithelial cuff and the accumulation of the same cells in the pocket indicate that they migrate through the epithelial lining under physiological conditions.

The amorphous substance between the cellular elements was identified as remnants of the gingival fluid.

On the basis of his findings the writer discusses the dynamics of the epithelial cuff, and its antibacterial defense mechanism.

RÉSUMÉ

ASPECTS PHYSIOLOGIQUES DU CULS-DE-SAC GINGIVO-DENTAIRE
ÉTUDE EXPÉRIMENTALE

Dans le but d'éclairer quelques aspects de la physiologie des culs-de-sac gingivo-dentaires, une série d'expériences sur l'animal a été effectuée. Chez trois chiens, 49 culs-de-sac cliniquement normaux ont été fermés au niveau du bord marginal pendant des périodes allant de 1 à 48 heures.

L'examen histologique des specimens a montré que les culs-de-sac contenaient des cellules épithéliales desquamées et des leucocytes neutrophiles dispersés dans une substance amorphe. La quantité du matériel recueilli augmentait avec la durée d'observation.

Les figures mitotiques trouvées sur toute la longueur du revêtement épithélial du cul-de-sac et la desquamation des cellules de la surface confirment le point de vue selon lequel le manchon épithélial est constamment renouvelé.

La présence de leucocytes neutrophiles à l'intérieur du manchon épithélial et l'accumulation de ces mêmes cellules dans le cul-de-sac indique qu'elles traversent le revêtement épithélial dans les conditions physiologiques.

La substance amorphe entre les éléments cellulaires a été identifiée comme étant les restes du liquide gingival.

S'appuyant sur ses résultats, l'auteur discute la dynamique du manchon épithélial et son mécanisme de défense antibactérienne.

ZUSAMMENFASSUNG

PHYSIOLOGISCHE ASPEKTE DER GINGIVALTASCHE
EINE EXPERIMENTELLE STUDIE

Um einige physiologische Aspekte bei der Gingivaltasche abzuklären, wurden eine Reihe von Tierexperimenten durchgeführt. An drei Hunden wurden 49 klinisch normale Taschen am Zahnfleischrand für die Dauer von 1 bis 48 Stunden verschlossen. Die histologische Untersuchung zeigte, dass die Taschen abgestossene Epithelzellen und neutrophile Leukozyten enthielten, die in einer amorphen Substanz eingelagert waren. Die Menge der in den Taschen enthaltenen Massen nahm mit verlängerter Beobachtungszeit zu.

Der Befund von Kernteilungsbildern in der gesamten Ausdehnung des Taschenepithels und die Desquamation der Oberflächenzellen unterstützen die Auffassung, dass der Epithelsaum ständig erneuert wird.

Die Anwesenheit neutrophiler Leukozyten im normalen Taschenepithel und ihre Anhäufung in der Tasche deuten darauf hin, dass diese Zellen unter physiologischen Verhältnissen durch das Taschenepithel hindurchwandert.

Die amorphe Substanz zwischen den zellulären Elementen konnte als Ueberrest der gingivalen Flüssigkeitsabsonderung erkannt werden.

Gestützt durch die erhobenen Befunde diskutiert der Verfasser die dynamischen Vorgänge im Epithelsaum und seinen antibakteriellen Verteidigungsmechanismus.

RESUMEN

CONSIDERACIONES FISIOLÓGICAS DE LA BOLSA GINGIVAL

ESTUDIO EXPERIMENTAL

Con el fin de aclarar algunos aspectos fisiológicos de la bolsa gingival se hizo una serie de experiencias en animales. Se selló el margen gingival de 49 bolsas gingivales clinicamente normales en tres perros sanos. El período de observación varió entre 1 y 48 horas.

El examen histológico del material acumulado en la bolsa mostró células epiteliales descamadas y leucocitos neutrófilos dispersos en una substancia amorfa. La cantidad de material obtenido aumentó con el tiempo de observación.

El hallazgo de figuras mitósicas todo a lo largo del tapiz epitelial y la descamación de las células superficiales apoyan la idea de que el "manguito" epitelial se renueva constantemente.

La presencia de leucocitos neutrófilos en el "manguito" epitelial normal y la acumulación de estas células en la bolsa indican que los leucocitos neutrófilos normalmente migran a través del tapiz epitelial.

El material amorfo encontrado entre los elementos celulares ha sido identificado como restos del fluido gingival.

En base a estos hallazgos el autor comenta la dinámica del "manguito" epitelial y su mecanismo de defensa antibacteriana.

REFERENCES

Brill N. and B. Krasse, 1958: The passage of tissue fluid into the clinically healthy gingival pocket. Acta odont. scand. *16:* 233.

Brill, N. and H. Björn, 1959: Passage of tissue fluid into human gingival pockets. Acta odont. scand. *17:* 11.

Brill, N. and R. Brönnestam, 1960: Immuno-electrophoretic study of tissue fluid from gingival pockets. Acta odont. scand. *18:* 95.

Evans, C. L., 1952: Principles of Human Physiology. 11. ed., London.

Gottlieb, B., 1921: Der Epithelansatz am Zahne. Dtsch. Mschr. Zahnheilk. *39:* 142.

Grant, D. A. and B. Orban, 1960: Leukocytes in the epithelial attachment. J. Periodont. *31:* 87.

Greulich, R. C., 1961: Epithelial DNA and RNA synthetic activities of the gingival margin. Preprint. Abstr., No. 118, I.A.D.R., March.

Hirt, Christine M., S. Hartl, and H. R. Mühlemann, 1955: The distribution of mitoses in the epithelium of the interdental papillae of the rat molar. J. Periodont. *26:* 229.

Lundquist, G. R., 1940: The zero crevice in the management of periodontal involvements. J. Amer. dent. Ass. *27:* 837.

McHugh, W. D., 1961: The development of the gingival epithelium in the monkey. Dent. Practit. *11:* 314.

Orban, B. J., H. Bhatia, J. A. Kollar, and F. M. Wentz, 1956: Epithelial attachment (the attached epithelial cuff). J. Periodont. *27:* 167.

Waerhaug, J., 1952: The gingival pocket. Odont. T. 60. Suppl. 1.

—»— 1956: Observations on replanted teeth plated with gold foil. Oral Surg. *9:* 780.

—»— 1957: Tissue reaction around acrylic root tips. J. dent. Res. *36:* 27.

—»— 1960: Current concepts concerning gingival anatomy. The dynamic epithelial cuff. Dent. clin. N. Amer., November.

Waerhaug, J. and H. Löe, 1957: Tissue reaction to gingivectomy pack. Oral Surg. *10:* 923.

—»— 1958: Effect of phenol camphor on gingival tissue. J. Periodont. *29:* 59.

Weinreb, M., 1960: Epithelial attachment. J. Periodont. *31:* 186.

Zander, H. A., 1956: A method for studying "the epithelial attachment". J. dent. Res. *35:* 308.