Keratinization Related to Premitotic Labeling and Inflammation of Gingiva and Alveolar Mucosa in Rhesus Monkeys

by

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IN SPITE OF SEVERAL investigations of the keratinized oral epithelia,[1-4] the relationship between keratinization and mitotic activity is controversial.[4, 5] It has also been postulated, without specific documentation, that inflammation reduces keratinization.[5, 6]

The purpose of the present study was to clarify the relationship between degrees of keratinization and rate of epithelial mitotic activity and/or inflammation.

In order to estimate mitotic activity as accurately as possible, H^3 thymidine premitotic labeling was used which necessitated that the study be done in animals. Rhesus monkeys were selected because of their close biologic similarity to humans.

MATERIALS AND METHODS

Radioautographs and histologic sections from three adult male monkeys used for another similar study[7] were used for the present investigation. The monkeys had mild chronic gingivitis. The processing of the radioautographs and the technique of examination have been described in a previous report.[7]

Keratinization was found only on the outer surface of the free gingiva and on the surface of the attached gingiva. No keratinized layer was found on the crevicular epithelium, the epithelial attachment or the alveolar mucosa. The study of degrees of keratinization therefore had to be limited to the areas that showed keratinization (the surface of the free gingiva and the alveolar mucosa). The degree of keratinization was quantitated by measuring the thickness of the surface keratin layer according to the method described by Glickman and co-workers,[2] using an adjustable ocular micrometer with a scale of 0-1000 (standardized and calibrated using an optical micrometer measurement slide†). The measurements of keratin layer thickness were recorded in microns.

In order to compare values for keratinization with epithelial labeling, the template aperture of the microscope was moved from the surface keratin to the basal cell layer directly under the keratin. Radioactive index for the epithelium (RAI) was calculated by dividing the number of labeled basal cells in high power fields (×1000) by the total number of basal cells in the same fields and multiplying by 100. An inflammatory index for the connective tissue underlying the epithelium in the same microscopic fields was computed by dividing the number of inflammatory cells by the total number of connective tissue cells in the fields and multiplying by 100. Detailed description of the boundaries of each of the two zones and the entire sampling techniques can be reviewed in the previous paper.[7]

Mean values for keratin thickness (KT), radioactive index (RAI) and inflammatory index (II) with standard deviation and standard error of the means were calculated on the basis of measurements and counts from approximately 1500 high power microscopic fields for each of the three variables. These fields were selected from radioautographs and histologic sections from 141 blocks containing teeth and periodontium from the three experimental monkeys.

FINDINGS

The mean thickness of the keratin layer covering the free gingiva was only one half of the thickness of keratin covering the attached gingiva (Table 1). The mean thickness of keratin covering the outer surface of both the free and attached gingiva was about 10 microns.

TABLE 1
Keratin Thickness (KT) of the Free and Attached Gingiva

Zone	*Mean (microns)*	*SE*
Free gingiva (C)	6.13	0.66
Attached gingiva (D)	15.65	0.71
Free and attached gingiva (C, D)	10.92	0.56

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TABLE 2
Analysis of Between Zones
Difference KT Means

Zones	*Means (Microns)*	*Difference*	*F*	*p*
$\bar{D}$–$\bar{C}$	15.84–6.57	9.27	222.20	$p < .001$

TABLE 3
Keratin Thickness of Subzones
of Attached Gingiva

Zone	*Mean (Microns)*	*S.E.*
D_1	13.5	0.11
D_2	18.4	0.19
D_3	15.3	0.07

TABLE 4
Analysis of Between Zones Difference of
KT Means of Subzones in Attached Gingiva

Zone	*Means*	*Difference*	*p*
$\bar{D}_2$–$\bar{D}_1$	18.4–13.5	4.9	$p < .01$
$\bar{D}_3$–$\bar{D}_2$	18.4–15.3	3.1	$p < .01$
$\bar{D}_3$–$\bar{D}_1$	15.3–13.5	1.8	$p < .01$

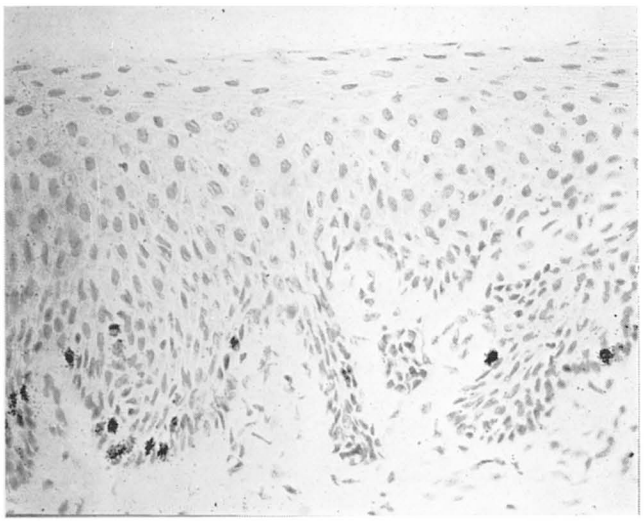

FIGURE 1. *Section from surface of free gingiva without a well defined surface layer of keratin. Note variation in isotope labeling from one rete peg to another. Original magnification X360.*

The difference in values for thickness of keratin covering the attached and the free gingiva was statistically significant ($p < .001$) (Table 2).

From the study of the sections, a general pattern of increasing keratin thickness appeared starting at the free gingival margin going to the middle of the attached gingiva, and then a gradual decrease in thickness until keratinization ceased at the border with the alveolar mucosa.

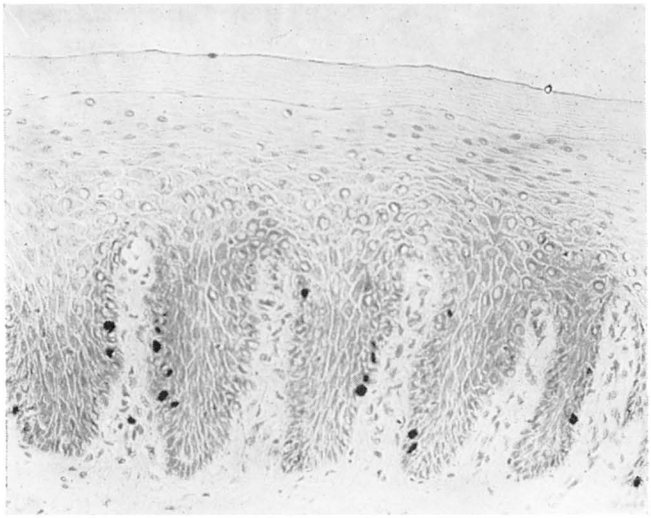

FIGURE 2. *Section from attached gingiva with a thick layer of surface keratin. Low incidence of labeling. Original magnification X300.*

Separate thickness measurements from the one third of the attached gingiva closest to the free gingiva (zone D_1), the middle one third (zone D_2) and the one third closest to the alveolar mucosa (D_3) (Table 3) also confirmed this trend. However, the differences in values for thickness between these subzones were statistically significant only between the middle (D_2) and the subzone close to the free gingiva (D_1) (Table 4). A total of 1459 microscopic fields from free and attached gingiva were sampled for keratin. Of these, 340 (24) did not have any measurable keratin layer (Fig. 1), while the thickness of keratin in the remaining fields varied from 1 to 57 microns (Fig. 2). The percentage of nonkeratinized fields was 54 for the free gingiva and 13 for the attached gingiva.

Correlation was tested between keratin thickness and premitotic labeling for the free and attached gingiva (Table 5). A significant negative correlation was found ($r = -0.334$, $p < .001$) both for each zone separately and for the two zones combined, indicating that

TABLE 5
Correlation Coefficient (r) Between KT,
and R.A.I. of Different Zones

Zone	*Number of Microscopic Fields Examined*	*r*	*t*	*p*
C	362	−0.254	−4.48	$p < .001$
D	1,097	−0.186	−6.20	$p < .001$
CD	1,459	−0.334	−13.22	$p < .001$

with increasing thickness of keratin, the premitotic labeling decreased significantly. Keratin thickness was also significantly negatively correlated with severity of inflammation (Table 6) ($r = -0.278$, $p < .001$), both for the separate and combined zones of the outer surface of the gingiva. This indicated that with increasing thickness of surface keratin there was less inflammation in the underlying connective tissues.

TABLE 6
Correlation Coefficient (r) Between KT and II

Zone	*Number of Microscopic Fields Examined*	*r*	*t*	*p*
C	362	−0.278	−5.41	$p < .001$
D	1,097	−0.233	−7.70	$p < .001$
CD	1,459	−0.278	−10.65	$p < .001$

Discussion

Keratinization, visible as a separate surface layer in light microscopic examination of histologic sections of the periodontium, was found only on the outer surface of the free and attached gingiva of rhesus monkeys. No attempt was made to study parakeratinization,[6] to study surface keratinization by smear techniques,[8] or to consider keratin formation in the granular cell layer.[9] The main reason for limiting the study to measurable surface keratin,[2] was to obtain reliable values, accurately localized, that could be compared to the mitotic and inflammatory activity in the tissues immediately corresponding to the surface keratin.

The findings in the present study are in accord with previous claims[5] of low mitotic activity in human gingiva asociated with heavy keratinization, but go against reported findings in rats[4] where apparently there is no relationship between thickness of keratin and mitotic activity.

The mitotic activity in the nonkeratinized alveolar mucosa is about twice as high as for the keratinized attached gingiva,[7] but these are tissues of different anatomical and physiologic characteristics so no statement can be made with regards to what role keratinization plays relative to the lower mitotic activity in the attached gingiva. From the comparison between premitotic labeling in the highly keratinized attached gingiva and the less keratinized free gingiva there appears to be a statistically significant inverse relationship between degree of keratinization and mitotic activity; but it is not possible from the present study to determine if this represents a cause and effect relationship.

As reported by other investigators,[5, 6] manifestations of inflammation increased with decrease in keratinization, but this does not establish a cause and effect relationship and refined experimental studies are needed to elucidate the relationship between keratinization and the disturbed tissue physiology in inflammation.

The reason for a heavier keratinization of the attached compared with the free gingiva is not obvious, and cannot be explained entirely on the basis of the difference in mechanical surface stimulation.

Summary and Conclusions

1. In rhesus monkeys, the average thickness of the surface keratin of the free gingiva was 6.13 microns, and for the attached gingiva 15.65 microns.

2. The outer surface of the free gingiva and the attached gingiva were the only periodontal structures showing histologic evidence of surface keratinization.

3. The thickness of the surface keratin was inversely correlated with premitotic labeling ($r = -0.334$, $p < .001$) and with degree of inflammation ($r = -0.278$, $p < .001$).

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