# The Role of Sulcular Environment in Controlling Epithelial Keratinization*

*by*
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IT HAS BEEN SHOWN that by altering the local environment the keratinization potential of epithelium may be changed.[1, 2] In a previous publication from this laboratory it was shown in monkeys, that when the sulcular epithelium was exposed to the oral environment it had the potential for keratinization.[3, 4] This finding was later corroborated by other publications.[5–7] Consequently although the sulcular connective tissue apparently carries the code for epithelial keratinization, it seems that the local environment is influencing the ultimate character of the epithelium lining in the sulcular area.

The purpose of the present study was to explore the role of the sulcular environment in controlling the keratinization of the outer surface gingival epithelium.

## MATERIALS AND METHODS

Three young adult Rhesus monkeys were used for this study. They had a generalized mild gingivitis, with minimal calculus and no significant bone loss. A prophylaxis was performed 1 week prior to the start of the experiment. The animals were anesthetized using sodium pentobarbital intraveneously, and the following surgical procedure was performed: Intrasulcular mucoperiosteal flaps were raised on the buccal aspect of individual teeth, without including the approximal papillae. The two vertical releasing incisions were carried beyond the mucogingival line. Once the flap was raised, an undermining incision through the periosteum was performed mesiodistally at the base of the flap. This incision of the periosteum allowed the flap to be pulled coronally. The marginal tissue was folded inwards, in such a way that the outer surface epithelium came in close contact with the tooth surface (Fig. 1). Atraumatic silk sutures No. 3-0 were used to maintain the folded flaps in position.

A total of 40 flaps were performed, covering observation periods of 1 hour, and 1, 3, 4, 7, 14, 21, 28 and 60 days. A plastic collar was used as a restraint to prevent the monkeys from ripping the tissues off during the experiment.

One hour prior to sacrifice the monkeys received an intraveneous injection of tritiated thymidine‖ (1 microcurie/gm of body weight, specific activity 6.7 Ci/mmole). The animals were sacrificed by exsanguination. After dissection the jaws were fixed in 10% formalin and decalcified in a saturated solution of EDTA at pH 7. After decalcification, the specimens were embedded in paraffin, and serially sectioned buccolingually at 6 $\mu$ intervals. One out of every four slides was processed for radioautography. The rest were stained with hematoxylin and eosin, Rhodamine B and Mallory's trichrome stains.

## RESULTS

### *Control Sections*

Sections from untreated teeth presented a normal arrangement of the marginal tissues (Fig. 2). The outer surface epithelium showed a definite keratinized epithelium, with areas of both orthokeratinization and parakeratinization. The sulcular area showed a nonkeratinized thin epithelium, with shallow rete pegs at the most coronal area.

### *Experimental Specimens*

*One Hour and 24 Hour Specimens.* Under low magnification the inverted flap can be seen clearly (Fig. 3). The keratinized gingiva is facing the tooth surface, keeping all the morphological characteristics of the outer surface gingiva and maintaining a close relationship with the tooth (Fig. 4a).

Under higher magnification, the keratinized surface of the epithelium is readily seen using Rhodamine B (Fig. 4b) or Mallory's trichrome (Fig. 4c). The folded epithelium shows definite thymidine uptake throughout this period (Fig. 5).

### *Three and Four Day Specimens*

In 3 and 4 days the epithelium facing the tooth is decreased in thickness, probably due to superficial desquamation (Fig. 6a). However, no inflammatory infiltrate is seen in the connective tissue and both epithelium and connective tissue show increased labeling (Fig. 6b). Rhodamine B and Mallory's trichrome specimens showed a discontinuous band of keratin at this stage.

### *Seven and Fourteen Day Specimens*

The epithelium facing the tooth gets thinner and changes its morphology showing wide and shallow rete pegs (Fig. 7a). Although superficial desquamation is evident, no positive stain with Rhodamine B or Mallory's trichrome is seen (Fig. 7b). Minimal thymidine uptake

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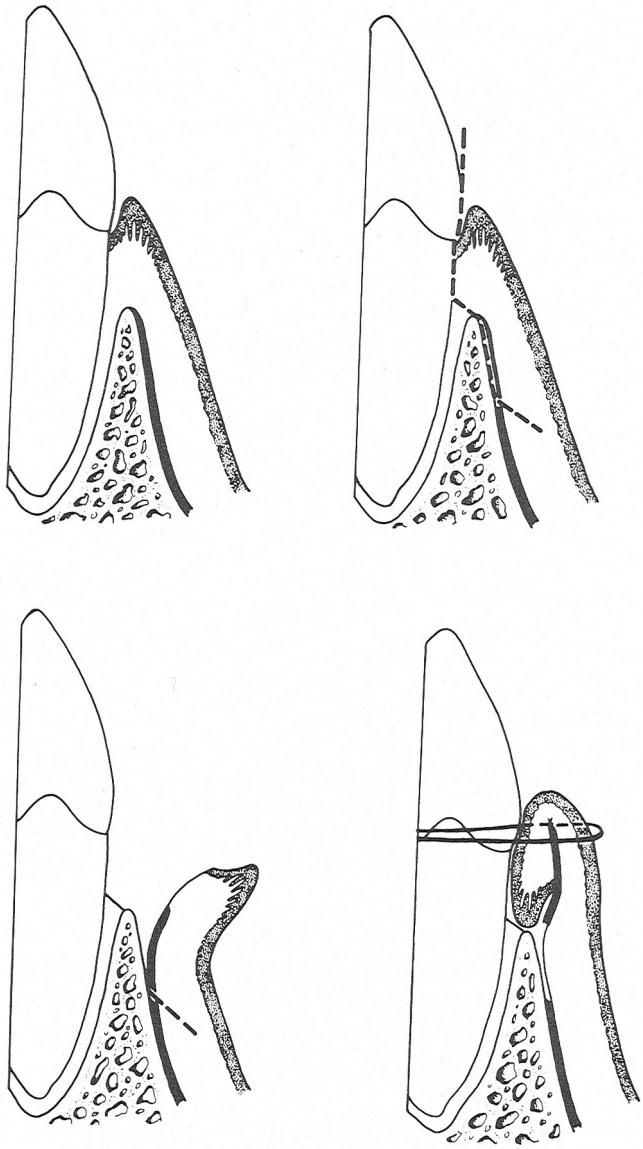

FIGURE 1. *Diagrams showing the procedure performed. See text for explanation.*

is seen (Fig. 7c). The connective tissue underlying the "new" sulcular epithelium does not display any inflammation. The previous "sulcular epithelium" is now embedded in the connective tissue. Total healing is seen at the line of fusion (Fig. 7a).

*Twenty-One, Twenty-Eight and Sixty Day Specimens*

The new sulcular epithelium has regained its regular morphology with very few broad rete pegs (Fig. 8a) and displays no keratinization when treated with Rhodamine B and Mallory's trichrome stains (Fig. 8b). The subepithelial connective tissue shows increased inflammation (Fig. 8a) and, associated with it, there is an increase in the thymidine uptake of the epithelium (Fig. 8c). Similar findings are observed in the 60 day specimens (Figs. 9a-c).

In a 28-day-old specimen, where the perfect adaptation of the flap to the tooth was lost, the healing and the reestablishment of a new sulcular epithelium took place by third intention. In this case the folded epithelium (remaining away from the tooth surface) maintained its keratinization while the restored sulcular epithelium did not show any keratinization (Fig. 10).

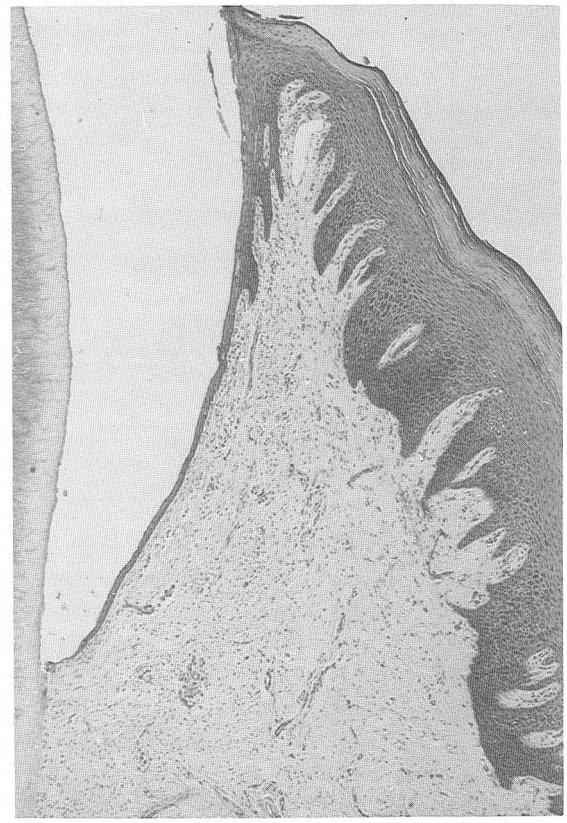

FIGURE 2. *Control section showing a keratinized outer surface gingival epithelium and a nonkeratinized thin sulcular epithelium (H & E, original magnification, × 40).*

## DISCUSSION

Sufficient evidence exists today to indicate that the sulcular epithelium has the potential for keratinization when moved away from the tooth surface environment.[3-7]

This evidence agrees with findings related to tissue specificity and genetic determination of epithelial differentiation.[8-10] The results by Karring et al.[8] experimenting with periodontal tissues concur with those reported for epidermal and oral tissues.[9, 10] As already mentioned[4] there is no difference in the composition of the connective tissue underlying the oral and the sulcular epithelium. As a consequence sulcular epithelium should have the potential to keratinize. Such a potential has been suggested earlier in the literature.[11, 12] Furthermore, this possibility recently has been associated with the eversion of the sulcular epithelium after orthodontic tooth separation in rats.[13]

If the potential is there, why doesn't the sulcular epithelium keratinize? Is it the sulcular environment that is inhibiting epithelial differentiation?

The findings from the present study seem to corrobo-

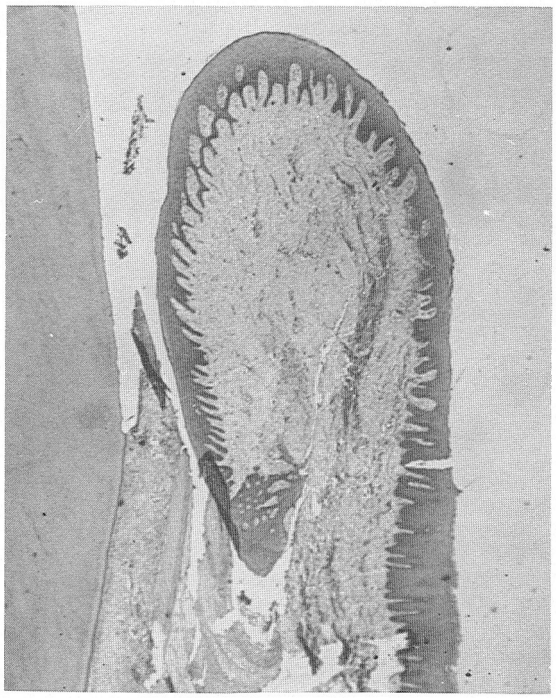

FIGURE 3. *Twenty-four hour specimen. The inverted flap is clearly distinguished. The kerantinized gingiva is facing the tooth surface (H & E, original magnification, × 12.5).*

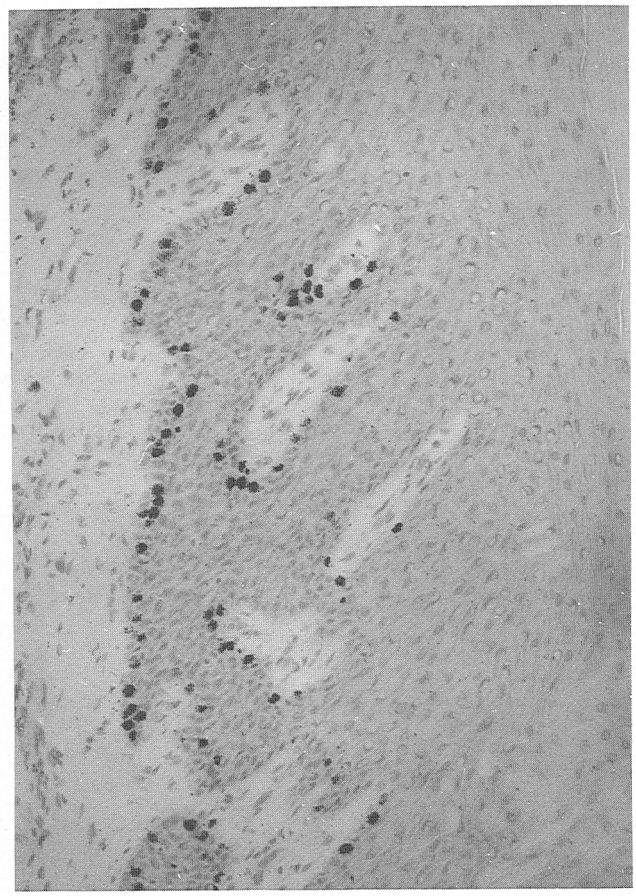

FIGURE 5. *Radioautograph showing thymidine uptake in the epithelium of a 24-hour specimen (original magnification, × 125).*

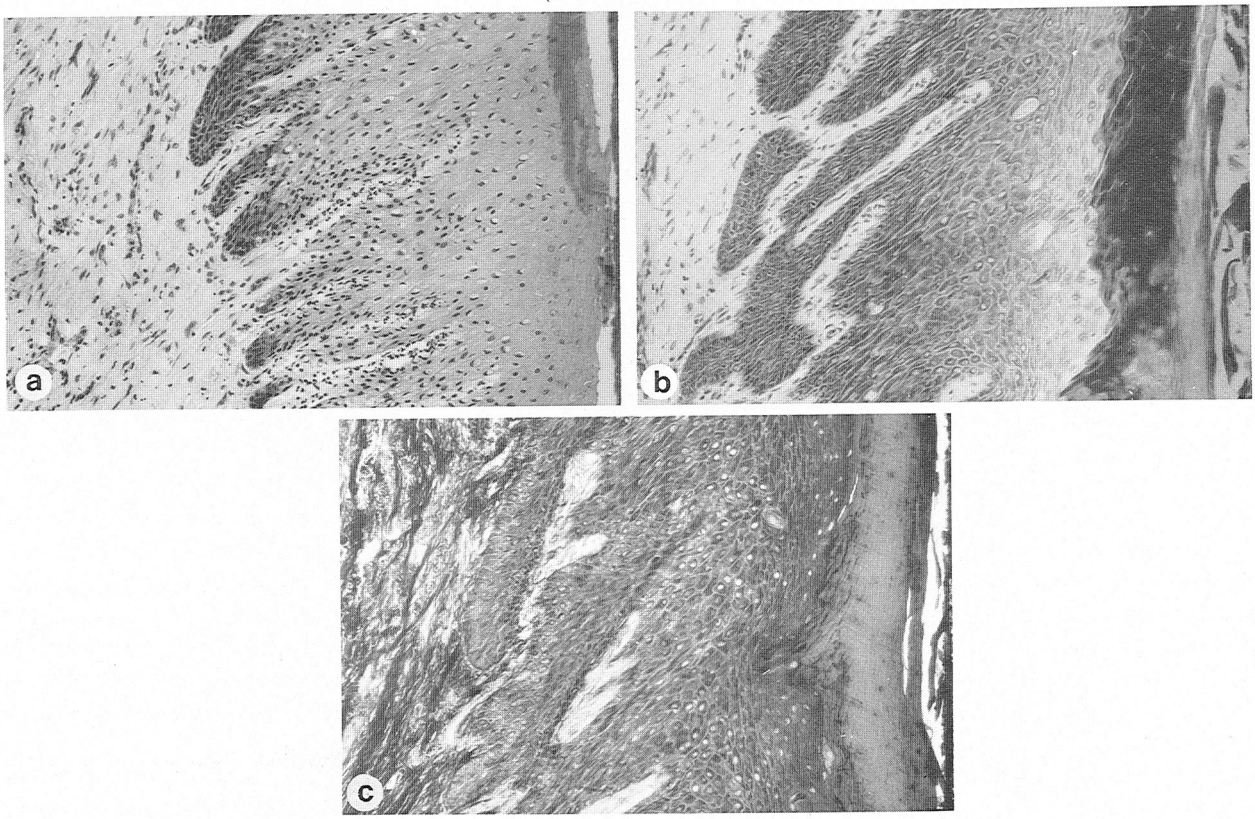


FIGURE 4. *Twenty-four hour specimens.* A. *The outer surface gingiva maintains a close relationship with the tooth surface (H & E, original magnification, × 125).* B. *A distinct keratinized epithelial surface is seen facing the tooth, with Rhodamine B stain (original magnification, × 125).* C. *Keratin layer stained with Mallory's trichrome (original magnification, × 125).*

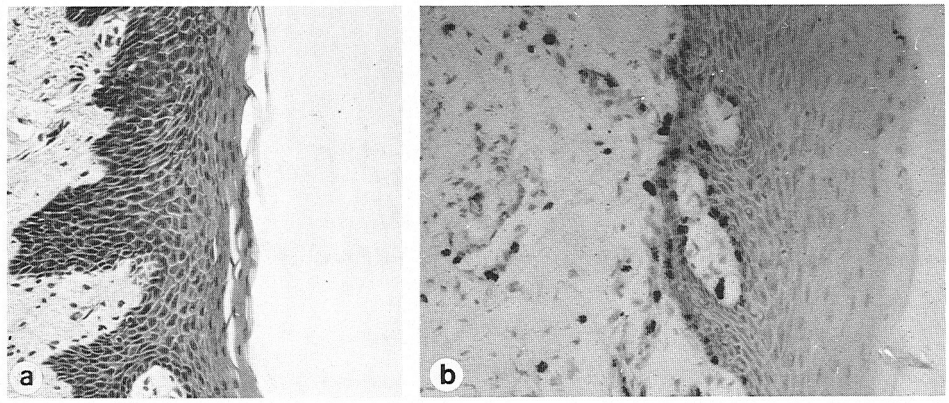


FIGURE 6. *Three days specimen.* A. *The epithelium facing the tooth is decreased in thickness, probably due to superficial desquamation. The connective tissue is free from inflammatory cells (H & E, original magnification, × 125).* B. *Radioautograph showing increased labeling both in the epithelium and connective tissue (original magnification, × 125).*

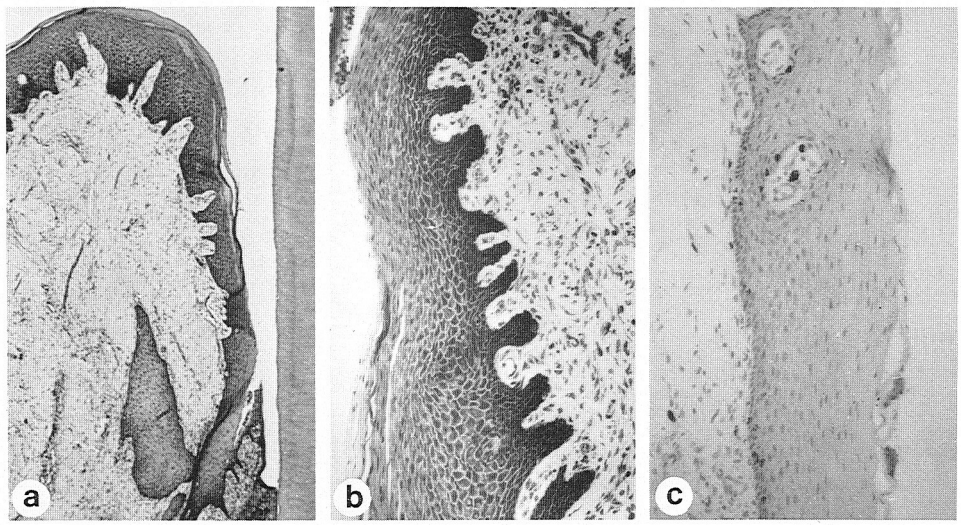


FIGURE 7. *Fourteen day specimen.* A. *The epithelium facing the tooth is thinner, with wide, shallow rete pegs. The former sulcular epithelium is now embedded in connective tissue (H & E, original magnification, × 40).* B. *Seven day specimen showing negative reaction to the Rhodamine B stain (original magnification, × 125).* C. *Fourteen day specimen showing decreased thymidine uptake (original magnification, × 125).*

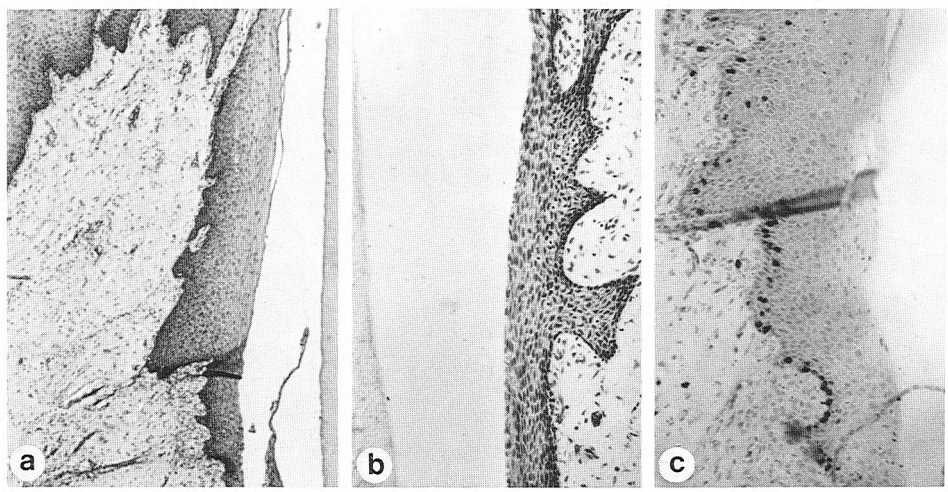


FIGURE 8. *Twenty-one day specimens.* A. *The new sulcular epithelium regains its regular configuration. The underlying connective tissue shows increased inflammation (H & E, original magnification, × 40).* B. *No keratinization is depicted when stained with Rhodamine B (original magnification, × 125).* C. *Radioautograph showing increased thymidine labeling in the epithelium (original magnification, × 125).*

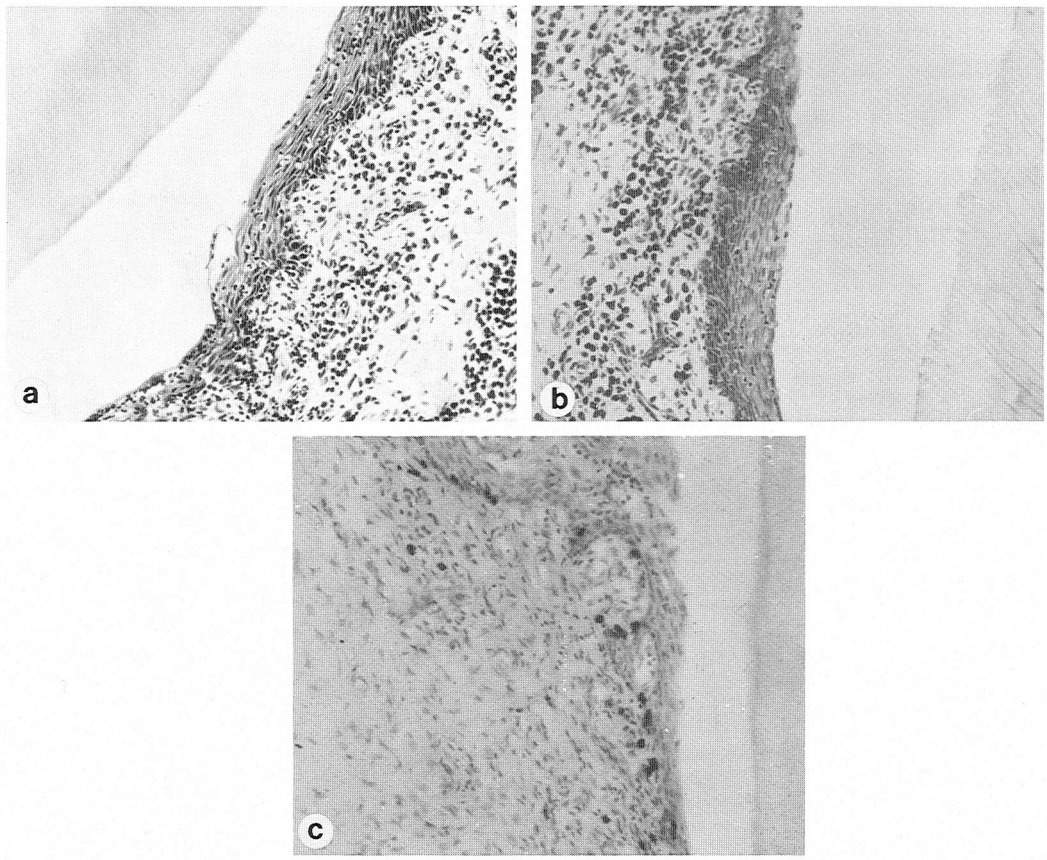


FIGURE 9. *Sixty-day specimen.* A. *A thin sulcular epithelium is noticed (H & E, original magnification × 64.5).* B. *No keratinization of the sulcular epithelium is depicted (Rhodamine B, original magnification, × 64.5).* C. *Radioautograph showing labeling of the sulcular epithelium (original magnification, × 64.5).*

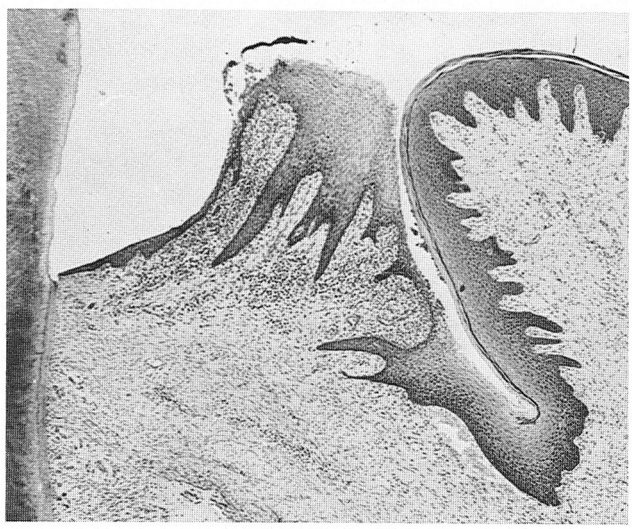

FIGURE 10. *Twenty-eight day specimen. Healing by third intention occurred due to poor flap adaptation. The folded epithelium maintained its keratinization (Rhodamine B, original magnification, × 40).*

rate this previously postulated assumption.[4, 6] The transformation of the outer surface epithelium to a thin nonkeratinized epithelium, devoid of deep and narrow rete pegs, when in contact with the tooth surface supports this hypothesis. Since the sulcular epithelium is subjected to constant irritation from bacterial plaque and its by-products, the epithelium may not develop full differentiation due to its increased turnover rate. Such premature desquamation has been suggested as a possible explanation for the lack of keratinization in some areas of the oral epithelium.[14] The increased thymidine label uptake observed in the sulcular epithelium of the 8 week specimens, is undoubtedly associated with increased inflammation in the underlying connective tissue. Interestingly enough, in the 28 day specimen showing poor flap adaptation to the tooth (Fig. 9) the folded epithelium maintained its keratinization. This is additional evidence that the contact to the tooth is responsible for the lack of keratinization of the epithelial tissues, probably due to the constant irritation they are subjected to.

This report presents further evidence to indicate that the sulcular environment determines the lack of keratinization of the epithelium. Important questions remaining to be explored and adequately answered are: can keratinization of the sulcular epithelium be achieved *in situ*? If this is possible, what is its clinical significance? If the hypothesis of a premature desquamation of the epithelium due to constant irritation from bacterial plaque is true, complete plaque removal should be able to reduce the turnover rate of the epithelial cells. This in turn, would allow the sulcular epithelium to fully differentiate. Contrary to the report by Kopczyk et al.[15] who showed a lack of keratinization of sulcular epithelium in dogs

after toothbrushing stimulation for 28 days, Hiatt[16] has shown the development of a keratinized sulcular epithelium in patients after long term use of an intracrevicular brushing technique. The short length of the experiment by Kopczyk et al.[15] might explain the lack of positive results. Whether a keratinized sulcular epithelium will act as a better barrier against the penetration of bacterial products is still to be determined.

## Summary and Conclusions

The influence of the sulcular environment on the keratinization of the outer surface gingival epithelium was tested in three young adult Rhesus monkeys. A total of 40 mucoperiosteal flaps were raised, and inverted so as to bring the outer surface epithelium in contact with the tooth, and were sutured. The experimental time intervals varied from 1 hour to 60 days. The monkeys received $H^3$ thymidine 1 hour prior to sacrifice. The material was prepared for histologic and radioautographic evaluation.

Results indicated that the outer surface epithelium changes its morphology to a nonkeratinized epithelium devoid of deep rete pegs when in close contact with the tooth, resulting in the anatomical characteristics normally seen in sulcular epithelium.

It is concluded that the sulcular environment has the capability of controlling the keratinizing potential of the outer surface gingival epithelium. It is further suggested that the constant irritation of bacterial plaque and its products may be responsible for the premature desquamation of the sulcular epithelium which in turn might not allow its full differentiation.

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# Announcement

UNIVERSITY OF KENTUCKY, COLLEGE OF DENTISTRY

The University of Kentucky, College of Dentistry announces the following continuing education course:

TITLE: "Tooth Movement"—Orthodontic/Periodontic Interrelationships

DATE: February 22, 1979

FACULTY: ORHAN C. TUNCAY, D.M.D., Assistant Professor, Department of Orthodontics, University of Kentucky.
DAVID C. VANDERSALL, D.D.S., M.S., Associate Professor, Department of Periodontics, University of Kentucky.

This course is designed to focus on the principles of treatment planning for limited tooth movement and to direct attention to the biological phenomenon associated with tooth movement. The participants will learn about the techniques of limited tooth movement, along with the physiologic and pathologic responses of the periodontium incidental to controlled orthodontic forces.

For further information contact: Ms. Alyce Ferguson, Coordinator, Continuing Education University of Kentucky, College of Dentistry, Lexington, Kentucky 40506.