# Keratinizing Potential of Sulcular Epithelium

*by*

RAUL G. CAFFESSE, D.D.S., M.S., DR. ODONT.*

THORKILD KARRING, D.D.S, DR. ODONT.

CARLOS E. NASJLETI, D.D.S.

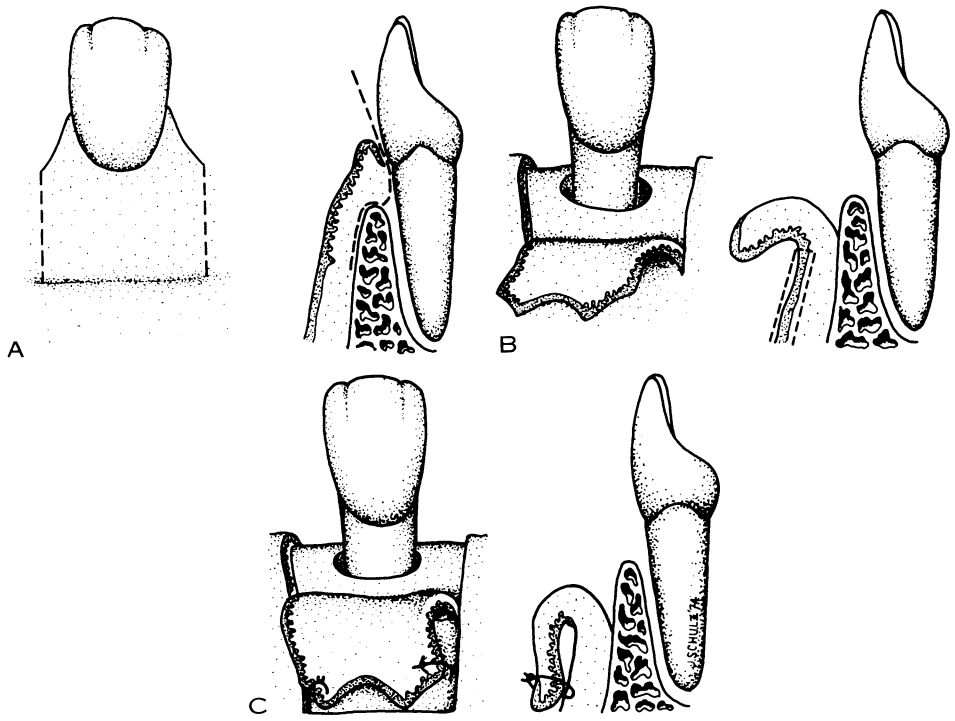
IT IS COMMONLY AGREED that the epithelium of the gingiva is either keratinized or parakeratinized while the sulcular epithelium does not contain a granular or a cornified layer.[1] When a flap of alveolar mucosa is transplanted to the attached gingiva it will maintain the normal tissue characteristics of alveolar mucosa; however, a collar of new attached gingiva will develop at the gingival margin, apparently from the reparative granulation tissue growing from the periodontal membrane.[2] Furthermore, it has been shown that the gingival connective tissue base will determine the characteristics of the overlying epithelium by carrying the genetic determinants for its specificity including keratinization.[3]

Since there is no apparent difference between the connective tissues underlying the sulcular and the outer gingival epithelium, and since after any gingival replacement procedures the periodontal membrane will be the common reparative source for both these tissues, it seems reasonable to assume that both epithelia, under the influence of the same connective tissue, may carry the same intrinsic potential for keratinization. The purpose of this study therefore, was to test the influence of the sulcular environment in determining the keratinization potential of the epithelium.

## MATERIALS AND METHODS

Three young adult Rhesus monkeys were used for the study. They had generalized mild gingival inflammation with minimal calculus, and sulcus depth no greater than 3 mm.

The animals were anesthetized with an intravenous injection of sodium pentobarbital. After two vertical incisions, intrasulcular mucoperiosteal flaps including the gingiva were raised by blunt dissection on the buccal aspect of individual teeth. The flap design included the approximal papillae to the tooth (Fig. 1A). Then, using a split thickness flap procedure, a connective tissue bed was prepared in the alveolar mucosa apical to the flap, by removing the outer epithelium and a thin layer of connective tissue (Fig. 1B). The elevated flaps were folded and sutured so that the sulcular epithelium became exposed to the oral cavity (Figs. 1C, 2A, B).




FIGURES 1A–C. *Diagrams showing the procedure performed. See text for explanation.*

* The University of Michigan School of Dentistry, Department of Periodontics, Ann Arbor, Mich. 48104 and the Veterans Administration Hospital, Ann Arbor, Michigan.

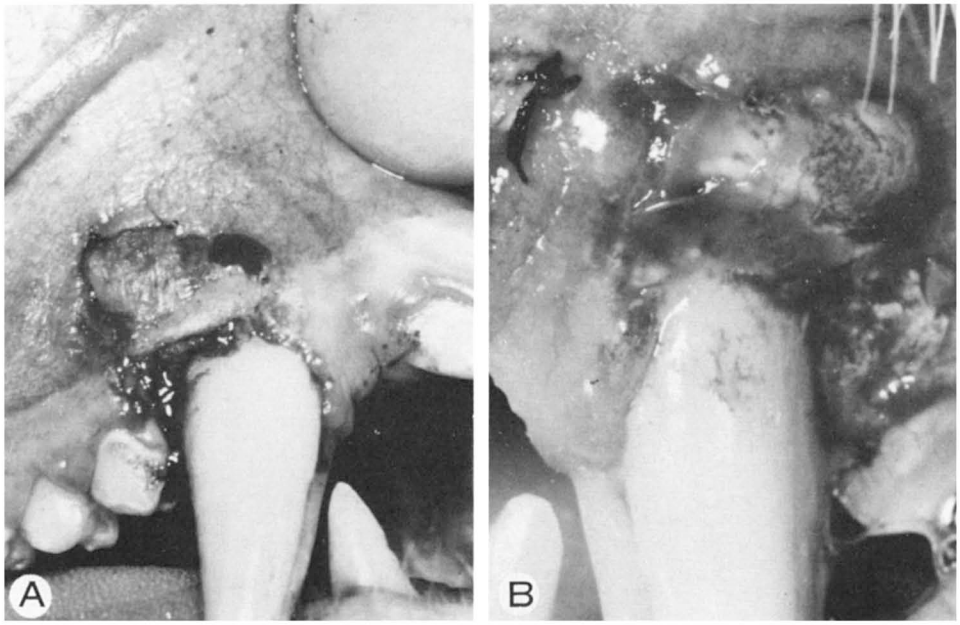



FIGURE 2A. *Flap appearance immediately after being folded out. The sulcular epithelium is exposed.* B. *Similar area 1 week after performing the procedure.*

In order to avoid the monkeys ripping the tissues off with their hands, a plastic collar was used as a restraint during the time of the experiment. Since they could not reach their mouths with their hands the regular food was provided in special containers placed in their cages at the level they could reach directly with the mouth.

A total of 24 flaps were performed in the three monkeys.

Soft tissue biopsies of the folded areas were taken from two monkeys after the following time intervals: 3 days, 5 days, 7 days, 2 weeks, 3 weeks, 4 weeks, and 8 weeks. The specimens were fixed, embedded in paraffin, and serially sectioned buccolingually at 6 $\mu$ intervals. The sections were stained with hematoxylin and eosin, and rhodamine B. Rhodamine B is a special stain specific for the demonstration of cornified tissue. Both orthokeratinized and parakeratinized epithelia will show a positive reaction, which is intense and precisely demarcated.[4]

In the third monkey, the folded flaps were performed according to a schedule which allowed for observation periods of 1, 12 and 24 hours and 3, 5 and 7 days. One hour before sacrifice the monkey received an intravenous injection of tritiated thymidine† (1 microcurie/gm of body weight, specific activity 6.7 Ci/millimole). The animal was sacrificed by exsanguination. The jaws were dissected, were fixed in 10% formalin and decalcified in a saturated solution of EDTA at a pH 7. After decalcification these 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, and rhodamine B.

† New England Nuclear, Boston, Mass.

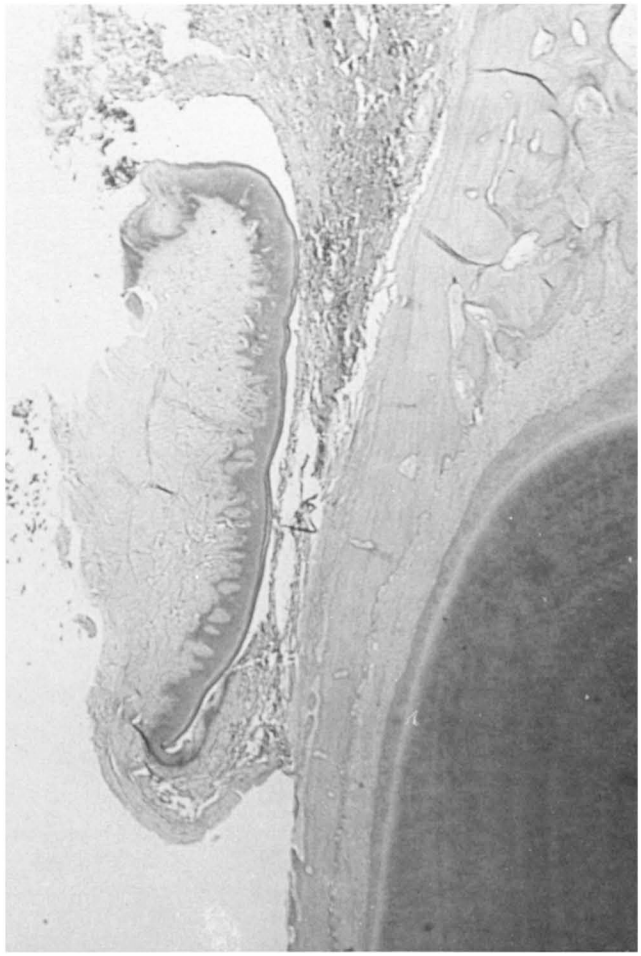


FIGURE 3. *One hour specimen. The folded flap is readily distinguished. Observe the remaining sulcular and junctional epithelium exposed to the oral environment. (H & E. Original magnification, × 30).*

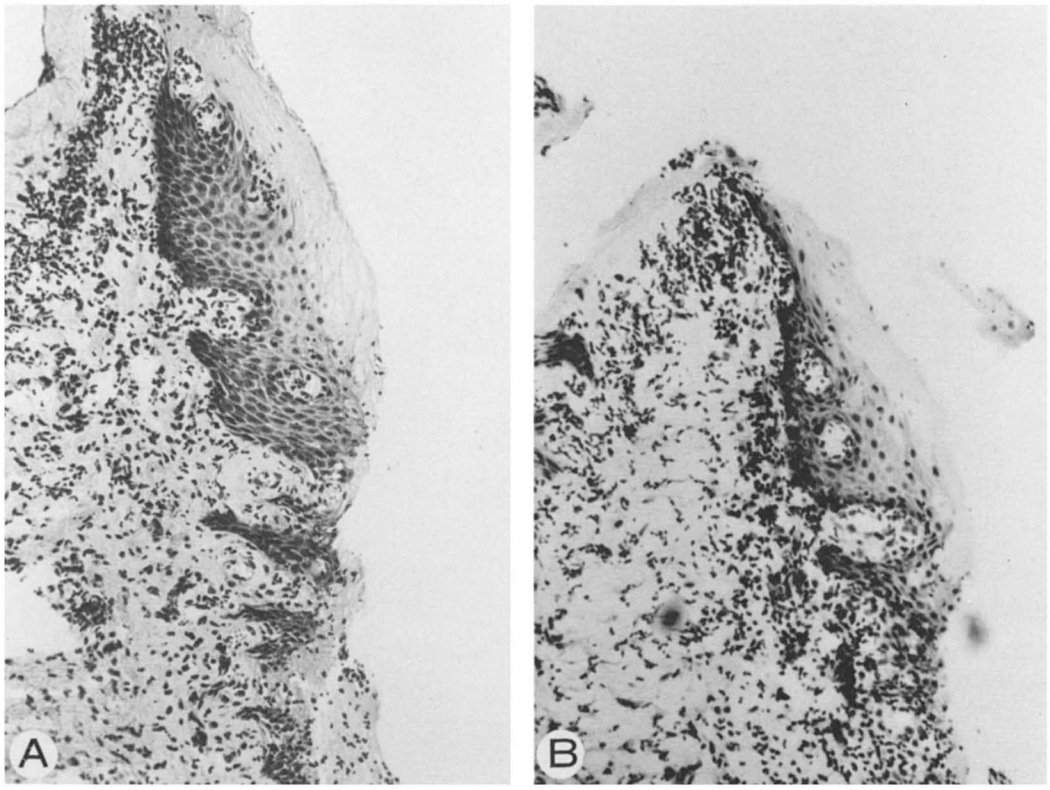



FIGURE 4A. *Twelve hour specimen. Remnants of the sulcular epithelium are seen. Its superficial portion shows necrosis and degeneration. Acute inflammatory infiltration is present (H & E. Original magnification, × 40).* B. *Twelve hour specimen. Radioautograph showing thymidine uptake throughout the basal cell layer (Original magnification, × 40).*

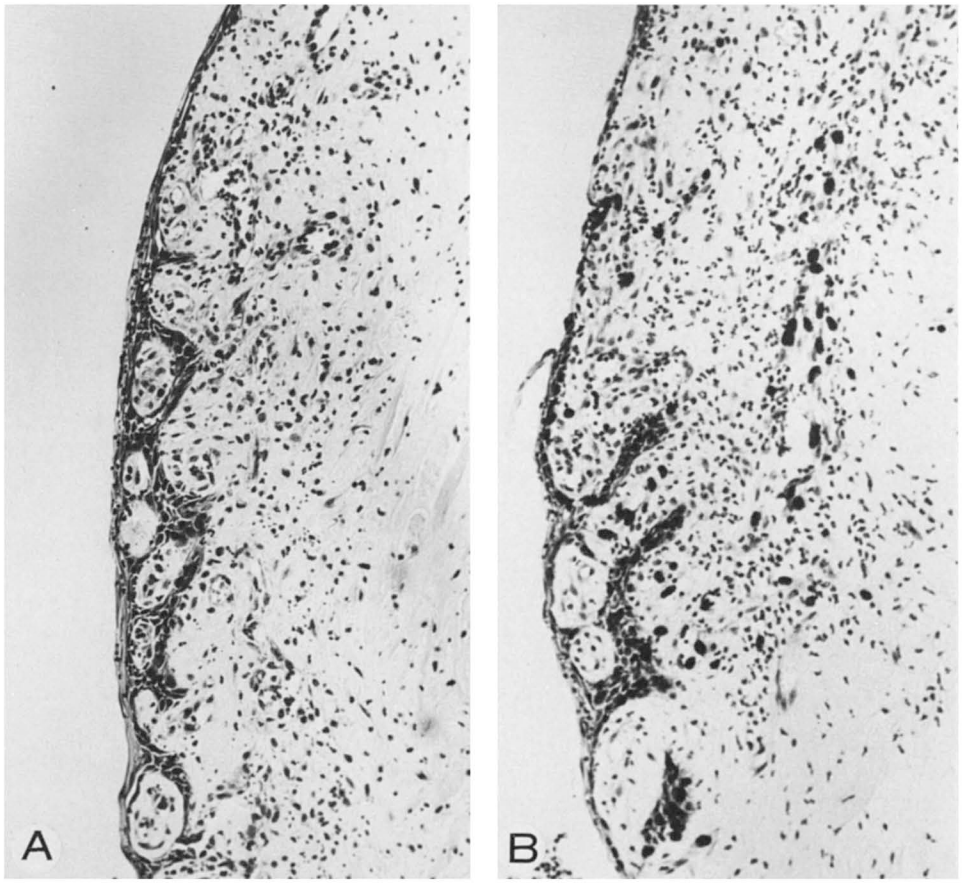



FIGURE 5A. *Three day specimen. A thin layer of epithelium is seen, due probably to the desquamation of the superficial layers (H & E. Original magnification, × 40).* B. *Three day specimen. Radioautograph showing increased labeling uptake throughout the remaining sulcular epithelium (Original magnification, × 40).*

## Results

*One and 12 Hour Specimens.* Under low magnification the folded tissues can be clearly observed (Fig. 3). H & E slides show the remnants to the sulcular epithelium (Fig. 4A). The superficial portion of the epithelium shows necrosis and degeneration. A severe acute inflammatory infiltrate is present. However, the basal cells showed thymidine uptake throughout (Fig. 4B). No positive reaction was obtained with rhodamine B.

*Twenty-four Hours to 7 Day Specimens.* The epithelium is thin, probably because the superficial cell layers have been desquamated (Fig. 5A). However, the remaining sulcular epithelium shows increased labeling (Fig. 5B), which seems to reach a peak in 5 days (Fig. 6). During this period the epithelium increases in thickness and also starts to migrate laterally trying to cover the exposed connective tissue (Fig. 7). Rhodamine B reaction is again negative. An acute inflammatory infiltrate is still present throughout the connective tissue.

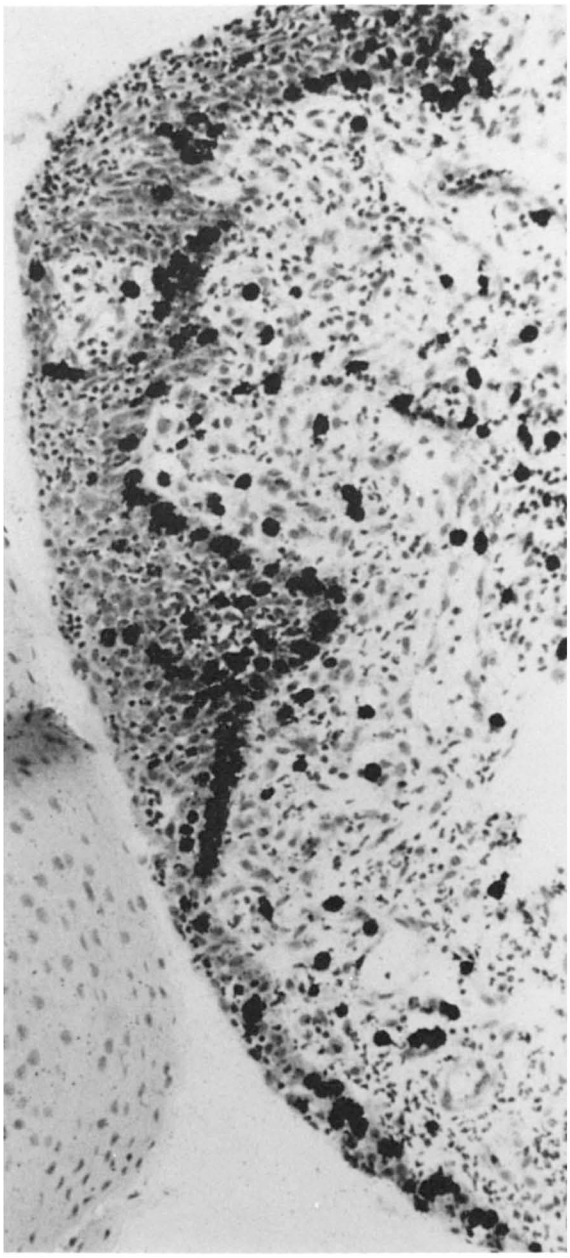


FIGURE 6. *Five day specimen. Radioautograph showing the labeling uptake depicted in the previous sulcular epithelium. The epithelium has increased its thickness. (Original magnification, × 40).*

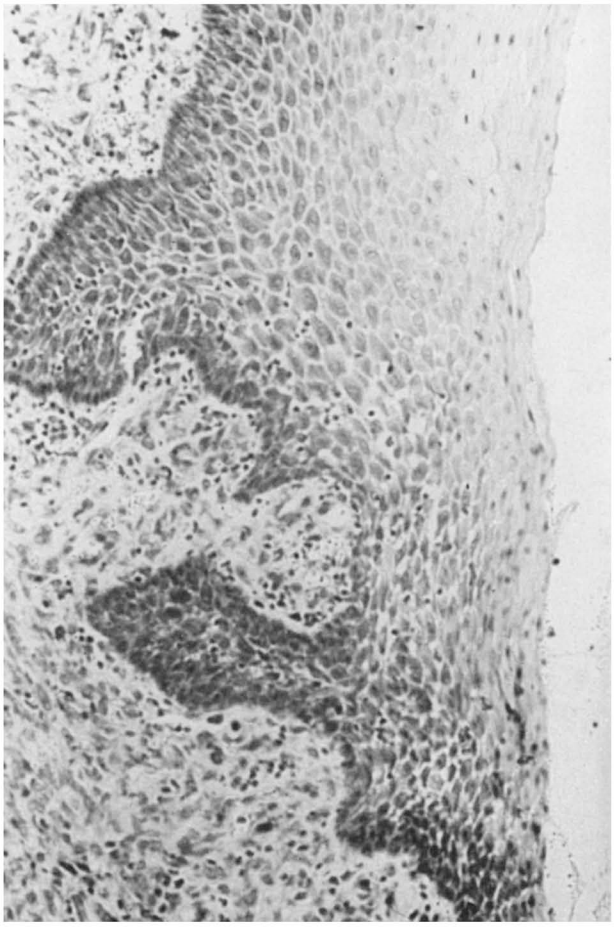


FIGURE 7. *Seven day specimen. The thickness of the epithelium has increased and rete pegs are being restored. (H & E. Original magnification, × 80).*

*Two and 3 Week Specimens.* The epithelial coverage is restored during this period. A new sulcular and junctional epithelium has been formed. It is possible to determine the area of junction between the original sulcular epithelium and the alveolar mucosa (Fig. 8A). The outer surface epithelium displays a normal arrangement, showing a multilayer pattern with intermingled areas of orthokeratinization and parakeratinization (Fig. 8B). There is a positive reaction with rhodamine B (Fig. 8C). Deep rete pegs are present, with an irregular or undulating basement membrane.

*Four and 8 Week Specimens.* In 4 and 8 week specimens findings similar to those just described can be seen. A new sulcular area has been developed and in most of the sections an intense chronic infiltrate is present underneath this new sulcular epithelium.

In one of the 8 week specimens, due to an area of improper healing at the junction between the original sulcular epithelium and the alveolar mucosa, it is possible to localize precisely the area of the folded sulcular epithelium (Fig. 9A).

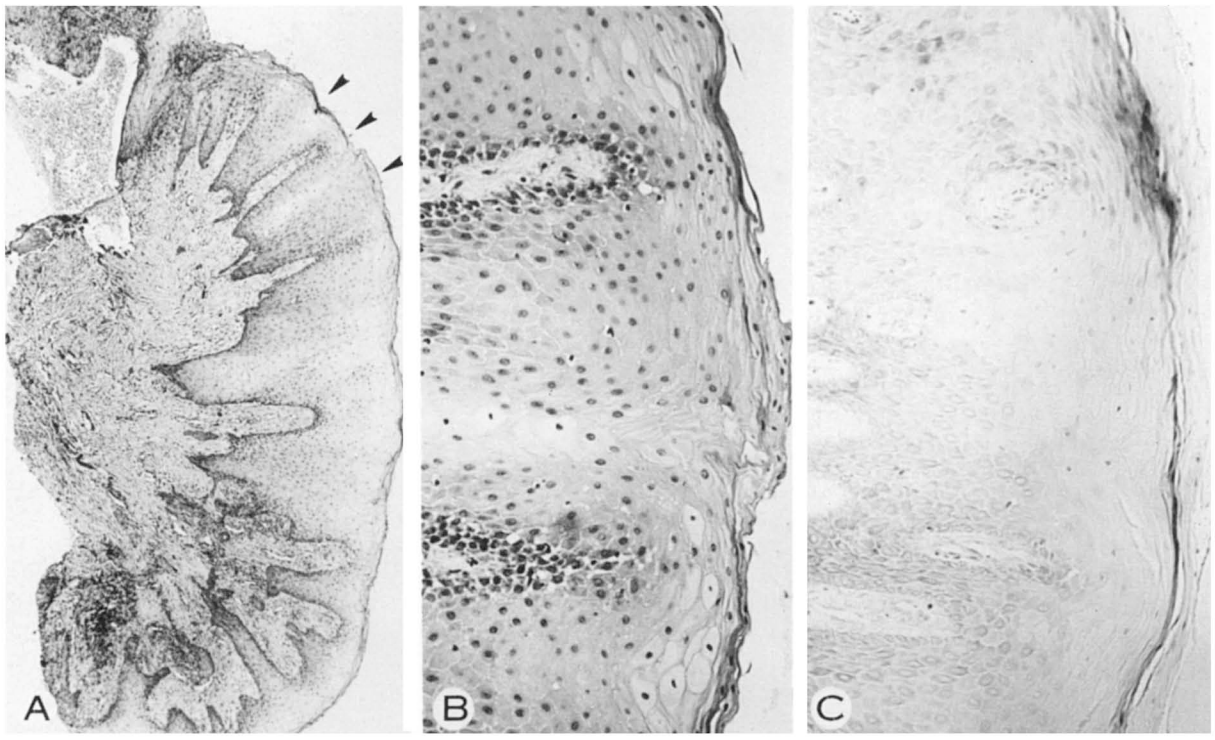



FIGURE 8A. *Three week specimen. The epithelium coverage is restored, and a new sulculus area has developed. The area corresponding to the previous sulcular epithelium* (arrows) *shows a normal arrangement (H & E. Original magnification, × 30).* B. *Three week specimen. Higher magnification of Figure A. Observe the multilayer pattern of the epithelium and its parakeratinization (H & E. Original magnification, × 60).* C. *Three week specimen. A beginning positive reaction to rhodamine B, can be observed at the superficial layers of the epithelium (Original magnification, × 60).*

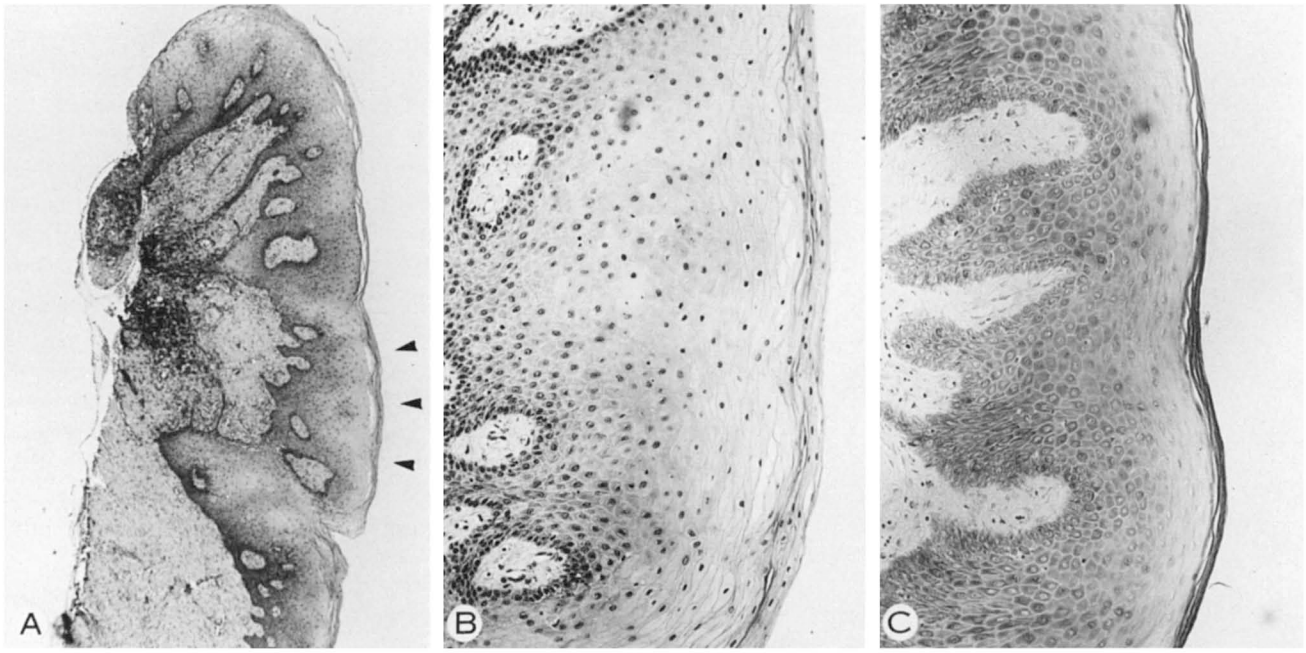



FIGURE 9A. *Eight week specimen. The improper healing at the junction between the original sulcular epithelium and the alveolar mucosa makes it possible to localize precisely the area of the folded epithelium* (arrows) *(H & E. Original magnification, × 30).* B. *Eight week specimen. Higher magnification of Figure A corresponding to the area derived from the original sulcular epithelium. Observe the normal characteristics of the surface epithelium (H & E. Original magnification, × 60).* C. *Eight week specimen. Very positive reaction to keratin can be seen on the surface (Rhodamine B. Original magnification, × 60).*

The alveolar mucosa migrated underneath the sulcular epithelium when covering the apical portion of the prepared bed. The outer epithelium derived from the original sulcular epithelium depicts all of the normal characteristics of the surface epithelium, (Fig. 9B) including a very positive reaction with rhodamine B (Fig. 9C).

## DISCUSSION

The present study has shown that when exposed to the environment of the gingival surface, the sulcular

epithelium develops into a keratinized squamous epithelium similar to that on the regular outer surface of the gingiva.

The labeling of the exposed sulcular epithelium following the administration of tritiated thymidine shows that it has maintained its vitality and its potential to proliferate after the exposure. The proliferation and subsequent development into a keratinized gingival epithelium could be followed, which excludes the possibility that the epithelial coverage of the flap had developed from neighboring areas.

The finding that the sulcular and outer gingival epithelium have the same potential for keratinization agrees with the assumption that both of these epithelia have the same connective tissue base. Why this same epithelium does not keratinize when acting as a boundary of the sulcus is speculative at the present time.

It may be that there are some unknown factors in the local environment that do not allow the epithelium to complete its differentiation. However, it also may be that the sulcular epithelium is not a surface epithelium, but only a crack or a cleft produced by an accident found in the local environment, that is the eruption of the tooth. If this should be the case the sulcular and the junctional areas would represent only a long rete peg split in the middle by the presence of the tooth. The oral epithelium is keratinized since it represents the outer surface of the masticatory mucosa (Fig. 10). The sulcular epithelium is not a surface epithelium, but is comparable to the deeper cell layers of the outer gingival epithelium. This view is supported by the similarity between the ultrastructure of the epithelium-enamel junction and that of the basement membrane, with the tooth representing the mesenchymal part of this interface.[5] Even the way the cells migrate during the turnover of these areas seem to agree with this unusual hypothesis.[6] This also may explain what has been shown, that after a prolonged and vigorous intrasulcular toothbrushing technique, it is possible to get keratinization of the sulcular epithelium.[7]

A similar situation is seen at the level of the interproximal gingival col, where mechanical stimulation can elicit keratinization.[8] However, in this particular case the authors were considering the surface gingiva of the interdental papillae and not the sulcular epithelium.

Finally, although the evidence for genetic predetermination of tissue characteristics is very strong, the possibility that the environment may play a role in the ultimate character of the sulcular epithelium cannot be overlooked. Whatever the reason is, the contact to the tooth appears to determine the lack of keratinization of the sulcular epithelium.

## Summary and Conclusion

It has been shown that the connective tissue base determines the epithelial surface characteristics. Although there is no apparent difference between the connective tissues underlying the sulcular and the surface gingival epithelium, the sulcular epithelium is not keratinized as is the gingival surface. The influence of the sulcular environment in determination of keratinization was explored in three adult Rhesus monkeys.

Twenty-four intrasulcular mucoperiosteal flaps were elevated beyond the buccal mucogingival border, were turned inside out and sutured to leave the sulcular epithelium exposed on the surface. Short term specimens (1 hour to 7 days) were obtained from one monkey which received $H^3$ thymidine 1 hour prior to sacrifice. Biopsies were obtained from the other two monkeys covering intervals of 1 week to 2 months.

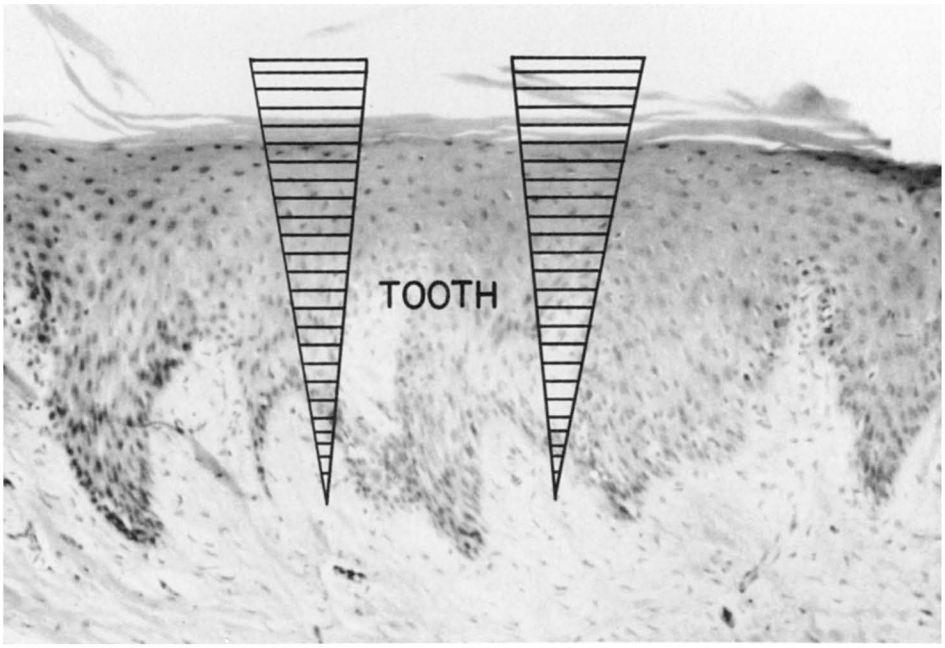



FIGURE 10. *Schematic representation showing why the sulcular and junctional areas might be considered as cracks or clefts in the continuity of the masticatory mucosa produced by the eruption of the tooth.*

The findings indicate that the sulcular epithelium has potential for keratinization. The contact to the tooth appears to determine the lack of keratinization of the sulcular epithelium.

## References

1. Löe, H.: Periodontium. H. Goldman, and W. Cohen, (eds), *Periodontal Therapy,* ed 4, pp 6–14. St. Louis, The C. V. Mosby Co., 1968.

2. Karring, T., Østergaard, E., and Löe, H.: Conservation of tissue specificity after heterotopic transplantation of gingiva and alveolar mucosa. *J Periodont Res* **6:** 283, 1971.

3. Karring, T., Lang, N. P., and Löe, H.: The role of gingival connective tissue in determining epithelial differentiation. *J Periodont Res* **10:** 1, 1975.

4. Clausen, F., and Dabelsteen, E.: Increase in sensitivity of the Rhodamine B method for keratinization by the use of fluorescent light. *Acta Pathol Microbiol Scand* **77:** 169, 1969.

5. Schroeder, H., and Listgarten, M.: *Fine Structure of the Developing Epithelial Attachment of Human Teeth.* S. Karger, Basel, 1971.

6. Skougaard, M., and Beagrie, G.: Renewal of gingival epithelium in marmosets (Callithrix Jacchus) as determined through autoradiography with thymidine $^{3}H$. *Acta Odont Scand* **20:** 467, 1962.

7. Hiatt, W.: *An Evaluation of the Present Status of Periodontal Therapy.* Continuing Education Course, 59th Annual Meeting of the American Academy of Periodontology, San Antonio, Texas. October 27, 1973.

8. Canton, M., and Stahl, S.: The effects of various interdental stimulators upon the keratinization of the interdental col. *Periodontics* **3:** 243, 1965.

# Announcement

### COLUMBIA UNIVERSITY SCHOOL OF DENTAL AND ORAL SURGERY

Columbia University School of Dental and Oral Surgery announces the following Continuing Education courses:

TITLE: Occlusion; A Periodontist's Approach to the Matter of Selective Grinding of the Natural Dentition as a Means of Modifying Occlusal Forces

DATE: April 29, 1977

FACULTY: ROBERT GOTTSEGEN, D.D.S., Professor and Director, Division of Periodontics; Past President, American Academy of Periodontology

A step-by-step procedure for selective grinding of the periodontally involved dentition will be described. The usefulness of this approach, in full or in part, to occlusal adjustment of less involved dentitions, to isolated lesions of occlusal traumatism and to general restorative dentistry, including extensive crown and bridge, will also be detailed. These steps will also serve as the guide posts for a stroll through the field of occlusion research and theory. Broad biological concepts will be discussed and related to clinical application. This course is designed for the clinician who is struggling to find some common denominator, some unifying thread of relevance in the confusing world of occlusion.

TITLE: Immunologic Aspects of Periodontal Disease

DATE: April 24, 1977

FACULTY: ROBERT GENCO, D.D.S., Ph.D.; Professor of Oral Biology and Periodontics; Director of Graduate Periodontology, State University of New York at Buffalo; and DANIEL FINE, D.M.D., Assistant Professor of Periodontics, Columbia University

An understanding of the etiologic aspects of periodontal disease is a prerequisite for the clinical practice of periodontics. Host responses, particularly immunologic responses related to periodontal disease, have far reaching implications with respect to understanding the diagnosis and treatment of periodontal disease. Recent advances in immunology have created an increasingly complex information base for the periodontist and periodontally oriented general practitioner.

This course will attempt to clarify the role of immunobiology in the periodontal disease process.

TITLE: A Day of Periodontics and Treatment Planning the Simple and Complex Periodontal Case with GERALD KRAMER (Sponsored by Columbia Periodontal Alumni, "Homecoming Day")

DATE: April 30, 1977

FACULTY: GERALD KRAMER, D.M.D., Professor and Chairman, Department of Periodontology, Boston University

A stimulating day of periodontal education in a unique format. AM: Dr. Kramer will present the evolution of his rationale and techniques for periodontal therapy and point out the changes in his approach over the years. PM: Match your treatment planning wits with the expert! Initial documentation of chosen cases will be shown from which each attendee will work up his own treatment plan. Dr. Kramer will present his solution. Then the actual long term result will be shown. All cases will have 10 years or more record. An exciting and valuable learning adventure.

For further information contact: Dr. Robert Gottsegen, Director, Continuing Dental Education, School of Dental and Oral Surgery, Columbia University, 630 West 168th Street, New York, N.Y. 10032.