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Evaluation of New Attachment Following Full Thickness Flap Procedure in Humans

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Abstract

EVALUATION OF NEW ATTACHMENT FOLLOWING FULL THICKNESS FLAP PROCEDURE IN HUMANS

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A study was made to investigate the biological possibility of obtaining new connective tissue attachment to periodontally diseased root surfaces following full thickness flap procedure. Reconstructive surgery was performed on seven teeth involved in advanced chronic periodontal disease. A mucoperiosteal flap was raised and the apical extent of existing subgingival calculus was marked with a notch made into the root just apical to the calculus. Following thorough instrumentation, sterile saline was applied to the root surface for five minutes, and the flap was repositioned and sutured. Four months later the teeth with attached periodontal tissues were removed and processed for histological analysis. None of the specimens demonstrated regenerating soft connective tissue or evidence of cementogenesis coronal to the notch. The base of the junctional epithelium extended from 0.55 to 2.40 mm below the coronal aspect of the notch. Therefore, at least among these seven specimens, the biologi-

cal possibility of obtaining new connective tissue attachment to diseased root surfaces following conventional full thickness flap procedures was not demonstrated.

LOMA LINDA UNIVERSITY

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Each person whose signature appears below certifies that this manuscript in his opinion is adequate, in scope and quality, in lieu of a thesis for the degree of Master of Science.

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INTRODUCTION

Prior to any discussion concerning healing of reconstructive periodontal therapy, definition of terms is necessary for clarity and understanding. In this text, reunion of connective tissues to root surfaces pathologically exposed in a periodontal lesions is termed "new attachment". "Re-attachment" is the reuniting of connective tissues and root surfaces which have been separated by surgery or trauma.

An abundance of literature is available regarding regeneration after various periodontal therapeutic techniques in humans. The healing of the periodontium has been evaluated utilizing periodontal probing, radiographs, re-entry procedures and microscopy after biopsy. Of the four methods, only microscopic examination will allow interpretation of achieved new attachment.

Procedures designed to produce connective tissue new attachment may be divided into several categories; closed curettage, flap procedures (open curettage), utilization of osseous grafts and utilization of acid application to root surfaces.

Closed curettage:

While new attachment procedures by means of curettage are thought to offer potential for regeneration, the amount

of new attachment that may be gained is uncertain (Kalwarf 1974). Schaffer & Zander (1953) performed a closed curettage procedure and reported obtaining "new attachment" in four out of six treated teeth. In this study, the apical extent of root planing was used to demarcate the original attachment level.

Flap procedures:

Several studies by Morris (1949, 1953, 1963) examined periodontal healing of surgically created defects in man at the level of a nick placed in the roots of the teeth studied at the depth of surgical separation. An initial resorption phenomenon along the experimental nicks was observed, followed later by connective tissue attachment through apposition of new cementum. Frank et al. (1972, 1974) also demonstrate a reattachment following flap procedure with root planing, at the apical extent of a groove placed at the level of the alveolar bone.

Utilization of osseous grafts:

Dragoo & Sullivan (1973) histologically evaluated the use of fresh autogenous iliac bone with hematopoietic marrow grafts in humans. They reported healing of chronic periodontal lesions with a "true attachment" which was defined as consisting of new bone, new cementum and new periodontal

ligament.

Bone regeneration and supposed new attachment following various osseous grafting procedures have also been reported (Froum et al. 1975, Hawley & Miller 1975, Ross & Cohen 1968, and Hiatt, Schallhorn & Aaronian 1978). These apparent regenerative successes have come under some criticism as a result of the studies by Caton & Zander (1976), Moskow, Karsh & Stein (1978), and Hiatt, Schallhorn & Aaronian (1978) which showed that an epithelial lining may persist post-operatively along the root surface adjacent to newly formed bone in the area of the original lesions.

Unfortunately, none of the above reports on curettage, flap procedures and osseous grafts has pre-treatment markings to delineate the original periodontal pocket. Therefore, conclusive evidence of new attachment is still lacking.

Acid demineralization:

There is evidence that treatment of the root surface via citric acid demineralization may improve the possibilities of obtaining connective tissue regeneration (Register 1973, Register & Burdick 1975, 1976). Stahl & Froum (1977) attempted citric acid conditioning in the treatment of six teeth in a patient, and did not observe any evidence of new attachment. However, a more recent study by Cole et al.

(1980) demonstrated different results. These authors used the apical extent of subgingival calculus to demarcate the apical extent of the original periodontal pocket in ten tooth surfaces from six patients. The surgical therapeutic procedures involved thorough instrumentation and five minutes of topical application of saturated citric acid. Four months post-operatively, block biopsies were examined histologically. Connective tissue regeneration, characterized by some deposition of new cementum, and more coronally, by tightly apposed soft connective tissue had occurred coronal to the notch in all specimens. The results demonstrate that regeneration of periodontal tissues to a root surface that has become denuded as a result of chronic, destructive periodontal disease and that has been covered by calculus is, in fact, a biological possibility. However, the study did not establish whether or not acid conditioning of the root surface is a prerequisite for new attachment. Thus, the question remains, could this same result have been obtained without the use of citric acid?

In designing a study to evaluate this question, utmost care must be taken to accurately assess the amount of regeneration. Initially, the extent of the original periodontal pocket must be marked. Utilization of the apical extent of root planing is not an acceptable marker since investigators

have shown that the healthy connective tissue attachment below the pocket is in many cases surgically separated during the operative procedure (Waerhaug 1955, Moskow 1962). It is also difficult to determine accurately the area of new attachment by evidence of cementogenesis since cementum formation may occur on intact cementum adjacent to surgically created defects (Listgarten 1972). Probably the most accurate marker which unequivocally delineates the extent of the periodontal pocket is calculus. Any connective tissue histologically found coronal to this level is unquestionably regeneration.

MATERIAL AND METHODS

Participants in this study included two men and four women, 35 to 62 years old, requiring a combination of periodontal and prosthetic treatment at the Loma Linda University School of Dentistry. An informed consent form was obtained from each patient. A total of seven condemned teeth (five incisors, one canine, and one bicuspid) scheduled for extraction were utilized (see Table 1). All of the tooth surfaces studied exhibited the following criteria;

1) extensive loss of soft connective tissue attachment and alveolar bone.
2) periodontal pockets at least 6 mm and with subgingival calculus.
3) absence of periapical pathology.

Experimental procedures:

Initially, oral hygiene instructions were given to each patient, and subgingival debridement of non-experimental teeth was accomplished. Two experimental teeth exhibiting severe mobility were splinted to adjacent teeth using orthodontic wire and acrylic. Each patient was then scheduled for surgical correction of the experimental periodontal lesion. At the surgical appointment the following procedures were executed;

A supragingival reference groove was placed with a No. ¼ round bur into dentin. The distance from the most apical portion of this groove to the gingival margin was measured to the nearest 0.5 mm using a calibrated periodontal probe. Full thickness, buccal and lingual flaps were reflected and soft tissues curetted from the wound. A notch was inscribed into the root surface (again using a No. ¼ round bur) in order to demarcate the apical extension of the calculus (see Fig. 1). In all cases the most coronal aspect of the notch corresponded to the most apical extent of the calculus. Care was taken to avoid horizontal overextension of the notch beyond the horizontal extension of the calculus. The distance from the calculus notch to the alveolar crest was recorded, as well as the depth of existing osseous defects (Figs. 1,2). The root surfaces were then planed in an attempt to remove all calculus and cementum. Care was taken to preserve the position of the notch during the root planing procedure.

Following root planing, the experimental teeth were treated with five minute topical application of cotton pledgets soaked with sterile saline. All teeth were then rinsed with water, and the flaps were replaced and tightly sutured.

The experimental surgery and post-operative treatment was carried out along with the regularly scheduled treatment plan, and was of minor inconvenience to the patients. Post-

operative care included administration of 250 mg oral tetracycline every six hours for seven days and twice daily rinses with 0.2% chlorhexidine gluconate during the entire four month post-operative period. Sutures were removed after one week, and subsequent oral hygiene and healing checks were performed every other week.

At the end of the four month post-operative period measurements were again recorded from the supragingival reference groove to the gingival margin in order to determine the amount of recession. The experimental teeth were then removed in block section. After removal of the specimens, the surgical areas were filled with freeze-dried homologous bone grafts, tightly sutured, and covered with an acrylic partial denture until healing was complete. The extraction procedure did not cause any functional disability or esthetic disfiguration.

Histological analysis:

The specimens were fixed in 10% neutral buffered formalin, decalcified in 5% formic acid, dehydrated in ethanol and embedded in paraffin. Longitudinal serial sections were made at 7 μm in an appropriate plane and stained with hematoxylin and eosin. Five step serial sections at 150 μm intervals, demonstrating a clearly discernible notch, were used for

histometric analysis. An additional slide from each specimen was stained with Gomori's Trichrome for confirmation of the extent of epithelialization.

Measurements were made using a calibrated ocular grid at 100 X magnification by placing the grid parallel to the root surface and moving it in a coronal or apical direction as necessary. Mean measurements were obtained for each specimen by averaging the values of the five step serial sections.

RESULTS

As shown in Table 1, notches were located from 0.5 to 2.5 mm above the pre-treatment crest of bone ($\bar{x}$ = 1.2± 0.8, Fig. 2). All of the specimens demonstrated an infrabony defect, ranging 1.0 to 4.5 mm in depth ($\bar{x}$ = 1.6±1.5, Fig. 2). The gingival margins showed recession during the treatment period ranging from 0.5 to 3.0 mm ($\bar{x}$ = 1.6±0.8 mm).

Histometrically, the mean width of the notch (CN-AN) was 0.70±0.17 mm. The base of the junctional epithelium was located apical to the coronal aspect of the notch in all the specimens extending from 0.55 to 2.40 mm (CN-JE). None of the specimens demonstrated regenerating soft connective tissue or evidence of cementogenesis coronal to the notch.

The root surfaces were planed to a mean distance of 1.27 mm below the notch (AN-RP). In five cases, the base of the junctional epithelium was coronal to the apical extent of root planing, whereas in one case it extended to a point below this level. Although the root surfaces had been carefully planed, small areas of remaining cementum were present on the treated surfaces in three of the seven specimens.

The alveolar bone extended coronal to the coronal extent of the notch in two of the specimens, and apical to it in four of the specimens. There was no evidence of ankylosis or root resorption.

Figure 3 represents a typical histological specimen (specimen #1), with a long, thin junctional epithelium extending to the depth of the notch.

The specimen demonstrating the longest junctional epithelium (specimen #2) is shown in Fig. 4. New cementum appears to have formed apical to the notch, and connective tissue reattachment has occurred to the apical extent of root planing.

Figure 5 is a diagramatic representation of the mean values obtained for the histological measurements.

DISCUSSION

This investigation was initiated to study the biological possibility of obtaining new connective tissue attachment in human teeth involved in chronic destructive periodontal disease of long duration. The results showed that following full thickness flap procedures, none of the specimens showed evidence of connective tissue regeneration along the root surface coronal to the notch.

In any study designed to accurately access the amount of regeneration following therapeutic procedures, it is of utmost importance to establish the exact dimensions of the original periodontal pocket. Cole et al. (1979) designed a method whereby subgingival calculus was utilized as a reference point. In that study a notch was placed within the confines of the concrement, and therefore the amount of new connective tissue attachment was measured from the apical extent of the notch. Criticism of this methodology has arisen in light of the possibility that the notch environment may somehow be conducive to new connective tissue attachment formation. Therefore, a modification of notch placement, whereby the coronal aspect of the notch corresponded to the apical extent of the calculus, was utilized in this study.

The suggestion that the notch would stimulate regenera-

tion must be questioned. Whereas in the Cole study where all specimens demonstrated connective tissue regeneration, in the present investigation 4 of the 7 specimens showed complete epithelialization of the notch. It is of interest to note, however, that reattachment of connective tissue to root planed tooth surface did occur apical to the notch. A mean reattachment of 0.25 mm above the apical extent of root planing was found. Such reattachment to the instrumented root surface agrees with the findings of Schaffer & Zander (1953) and Frank et al. (1974).

The requirement that subgingival calculus must be present limits the number of specimen candidates for study. Also, the demands of the block biopsy technique limit the experimentation to those teeth which have been condemned. For these reasons, only a small number of specimens were used. Osseous defects were present on all surfaces studied, although none of the notches were placed subcrestally. Most of the specimens, however, had notches placed in very close proximity to the bone, and could be considered suitable candidates for new attachment attempts. For example, the specimen of Fig. 4 appears to have good potential for regeneration, but resulted in a long junctional epithelium. Likewise, none of the other specimens showed new attachment. These results are opposite to the findings by Cole et al. (1980). A similar experi-

mental design was used in that study except that the experimental root surfaces were treated with five minute application of citric acid at the time of surgery. Histologic examination revealed new connective tissue attachment in 10 out of 10 specimens. A close comparison of the case selection of the present study and the study by Cole does not indicate that the cases selected in the present study should have had any less potential for new attachment. In fact, out of the 10 treated surfaces of Cole, 6 were strict supragingival lesions. Still, new attachment was accomplished in all of them. The notch was placed within calculus in the Cole study, whereas it was placed apical to the calculus in the present study. Thus, a greater regenerative potential would seem to exist in the more apically positioned notch of the present study. This was not found to be the case, however, since all of the Cole specimens demonstrated complete regeneration within the notch, and none of the present specimens showed complete notch regeneration.

Certainly, direct comparison between the results of the present study and the study by Cole et al. (1980) should be made with extreme caution. The specimens originated from different patients and the surgical and post-operative procedures were performed by different operators. In spite of all reservations about possible difference between the

studies, the clearcut difference in results suggest that citric acid conditioning of the root surfaces facilitate new attachment and that corresponding results may not be possible without acid treatment.

This suggestion has been demonstrated in dogs by Crigger et al. (1978), where furcation defects were treated surgically with and without citric acid application. After six weeks healing, all of the non-acid treated teeth revealed a patent epithelialized furcation defect, whereas 21 of 24 acid-treated furcation showed complete connective tissue closure of the furcation.

In conclusion, then, it must be stated that although attempts at new attachment were unsuccessful in these seven specimens, this does not mean that new attachment cannot be accomplished with conventional techniques. Given better conditions, this may still be possible: however, it is not likely that new attachment regularly occurs after clinical procedures. Thus, the "gain of attachment" which has been reported in clinical studies (Ramfjord 1973, Rosling et al. 1976) is most likely a tight adaptation of junctional epithelium. This would tend to support the suggestion by Listgarten & Robinson (1976) to utilize the term "probing depth" in place of "pocket depth" for these clinical studies. Also, it would seem advisable to introduce the term "probing level"

as replacement for "attachment level" when discussing clinical measurements.

Table 1. Histometric evaluation of periodontal regeneration four months after corrective surgery. Measurements in millimeters.

Histologic reference points

GM=Gingival margin
CN=Coronal extent of notch
AN=Apical extent of notch
JE=Apical extent of junctional epithelium
BC=Bone crest
RP=Apical extent of root planing

Surfaces

L=Lingual
MB=Mesiobuccal
DB=Distobuccal
D=Distal

Specimen number	Tooth number, surface	Notch to bone crest*	Depth osseous defect*	Gingival recession*	GM-CN	CN-JE	CN-BC	CN-AN	CN-RP	Cementum left on root planed surface
1	7 L	**	**	1.0	4.26	-0.70	1.38	0.53	-1.01	-
2	6 MB	-1.0	1.0	0.5	7.35	-0.78	-0.55	0.75	-1.76	+
3	9 MB	-0.5	1.0	1.5	2.17	-0.55	-0.55	0.89	***	-
4	7 MB	-1.5	1.5	1.5	2.34	-1.34	-1.88	0.44	-1.95	+
5	8 L	-2.5	1.0	2.0	2.44	-0.61	-1.24	0.80	-1.20	-
6	7 D	-1.0	4.5	3.0	2.35	-2.40	****	0.66	-0.88	-
7	13 DB	-0.5	0.5	1.5	3.16	-0.69	1.23	0.83	-0.83	+
Mean		-1.2	1.6	1.6	3.44	-1.02	-0.27	0.70	-1.27	
Std. deviation		±0.8	±1.5	±0.8	±1.87	±0.66	±1.31	±0.17	±0.47	

*=Clinical measurements
**=Measurement not taken
***=Could not be determined due to tangential sectioning
****=Could not be determined due to bone removal during biopsy

REFERENCES

Caton, J. & Zander, H. 1976. Osseous repair of an infrabony pocket without new attachment of connective tissue. Journal of Clinical Periodontology 3:54-58

Cole, R., Crigger, M., Bogle, G., Egelberg, J. & Selvig, K. 1980. Connective tissue regeneration to periodontally diseased teeth. Journal of Periodontal Research 15: 1-9

Crigger, M., Bogle, G., Nilveus, R., Egelberg, J. & Selvig, K. 1978. The effect of topical citric acid application on the healing of experimental furcation defects in dogs. Journal of Periodontal Research 13:538-549

Dragoo, M. & Sullivan, H. 1973. A clinical and histological evaluation of autogenous iliac bone grafts in humans: Part I. Wound healing 2 to 8 months. Journal of Periodontology 44:599-613

Frank, R. & Cimasoni, G. 1974. Ultrastructural study of epithelial and connective tissue reattachment in man. Journal of Periodontology 45:626

Froum, S., Thaler, R., Scopp, E. & Stahl, S. 1975. Osseous autografts II. Histologic responses to osseous coagulum-bone blend grafts. Journal of Periodontology 46:656-661

Hawley, C. & Miller, J. 1975. A histologic examination of free osseous autograft. Journal of Periodontology 46: 289-293

Hiatt, W., Schallhorn, R. & Aaronian, A. 1978. The induction of new bone and cementum formation IV. Microscopic examination of the periodontium following human bone and marrow allograft, autograft and nongraft periodontal regeneration procedures. Journal of Periodontology 49: 495-512

Kalkward, K. 1974. Periodontal new attachment without the placement of osseous potentiating grafts: Literature Review. Periodontal Abstract 22:53

Listgarten, M. 1972. Electron microscopic study of the junction between surgically denuded root surfaces and regenerated periodontal tissues. Journal of Periodontal Research 1:68-90

Listgarten, M. & Robinson, P. 1976. Periodontal probing and the relationship of the probe type to periodontal tissues. Journal of Periodontology 47:511-513

Morris, M. 1949. Reattachment of periodontal tissue. Oral Surgery, Oral Pathology and Oral Medicine 2:1194-1198

Morris, M. 1953. The reattachment of human periodontal tissues following surgical detachment: A clinical and histological study. Journal of Periodontology 24:220-228

Morris, M. & Thompson, R. 1963. Healing of human periodontal tissue following surgical detachmen: Factors related to the deposition of new cementum of dentin. Periodontics 1:189-195

Moskow, B. 1962. The response of the gingival sulcus to instrumentation: A histologic investigation. Journal of Periodontology 33:282-291

Moskow, B., Karsh, F. & Stein, S. 1978. Histologic assessment of healing of human periodontal defect following autogenous bone graft. Journal of Dental Research 57: Special issue A, #106, p. 100

Ramfjord, S., Nissle, R., Shick, R. & Cooper, H. 1968. Subgingival curettage versus surgical elimination of periodontal pockets. Journal of Periodontology 49:167-175

Register, A. 1973. Bone and cementum induction by dentin demineralized in situ. Journal of Periodontology 44: 49-54

Register, A. & Burdick, F. 1975. Accelerated reattachment with cementogenesis to dentin, demineralized in situ. I. Optimum range. Journal of Periodontology 46:646-655

Register, A. & Burdick, F. 1976. Accelerated reattachment with cementogenesis to dentin, demineralized in situ. II. Defect repair. Journal of Periodontology 2:211-217

Rosling, B., Nyman, S., Lindhe, J. & Jern, B. 1976. The healing potential of the periodontal tissues following different techniques of periodontal surgery in plaque free dentitions. A two year study. Journal of Clinical Periodontology 3:233-250

Ross, S. & Cohen, D. 1968. The fate of a free osseous tissue autograft. A clinical and histologic case report. Periodontics 6:141-151

Schaffer, E. & Zander, H. 1953. Histologic evidence of reattachment of periodontal pockets. Parodontology 7: 101-107

Stahl, S. & Froum, S. 1977. Human clinical and histologic repair responses following the use of citric acid in periodontal therapy. Journal of Periodontology 48:261-266

Waerhaug, J. 1955. Microscopic demonstration of tissue reaction incident to removal of subgingival calculus. Journal of Periodontology 26:26-29

LEGENDS

Fig. 1. Palatal aspect of maxillary lateral incisor, demonstrating the subcalcular notch (N), bone crest (BC) and subgingival calculus (CAL).

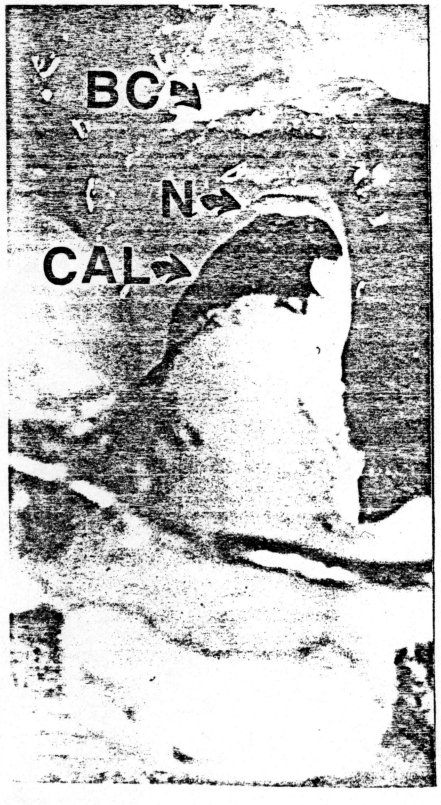
Fig. 2. Means of clinical recordings during surgery in mm;
CAL = subgingival calculus,
CN = coronal extent of subcalcular notch,
BC = bone crest,
FD = fundus of osseous defect.

Fig. 3. Diagramatic representation of mean post-operative histologic measurements in mm;
GM = gingival margin,
CN = coronal extent of subcalcular notch,
AN = apical extent of subcalcular notch,
JE = apical extent of junctional epithelium,
RP = apical extent of root planing,
BC = bone crest.

Fig. 4. Specimen number 7. Typical healing response, demonstrating long, thin junctional epithelium. The junctional epithelium extends through most of the depth of the notch (JE). Reattachment of connective tissue has occurred coronal to the apical extent of root planing (RP). BC = bone crest. Hematoxylin and eosin. Original magnification 5X.

Fig. 5. Specimen number 2.
A. Low power view showing a very long junctional epithelium, the apical extent of junctional epithelium (JE), and bone crest (BC). Gomori's trichrome stain. Original magnification 4X.

B. Magnification of notch area, confirming the location of apical extent of junctional epithelium (JE) in relation to the location of the coronal aspect of the notch (CN). Note new cementum (NC) apical to the notch. Original magnification 16X.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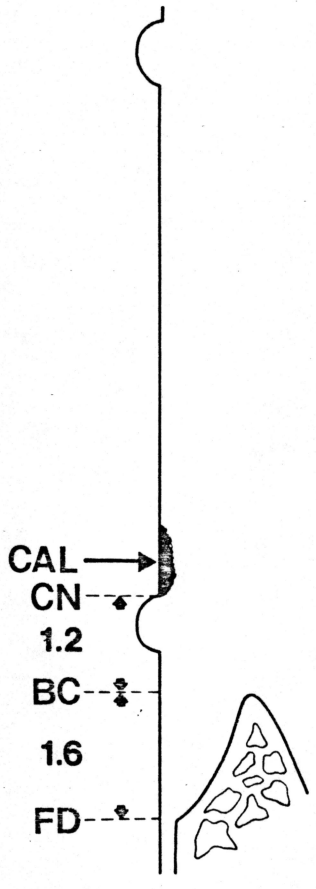
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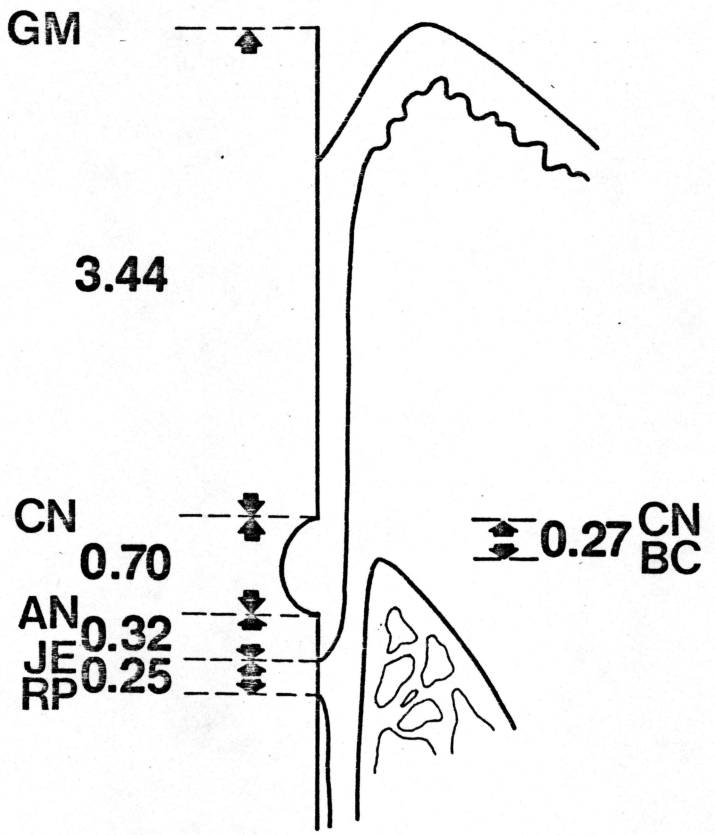
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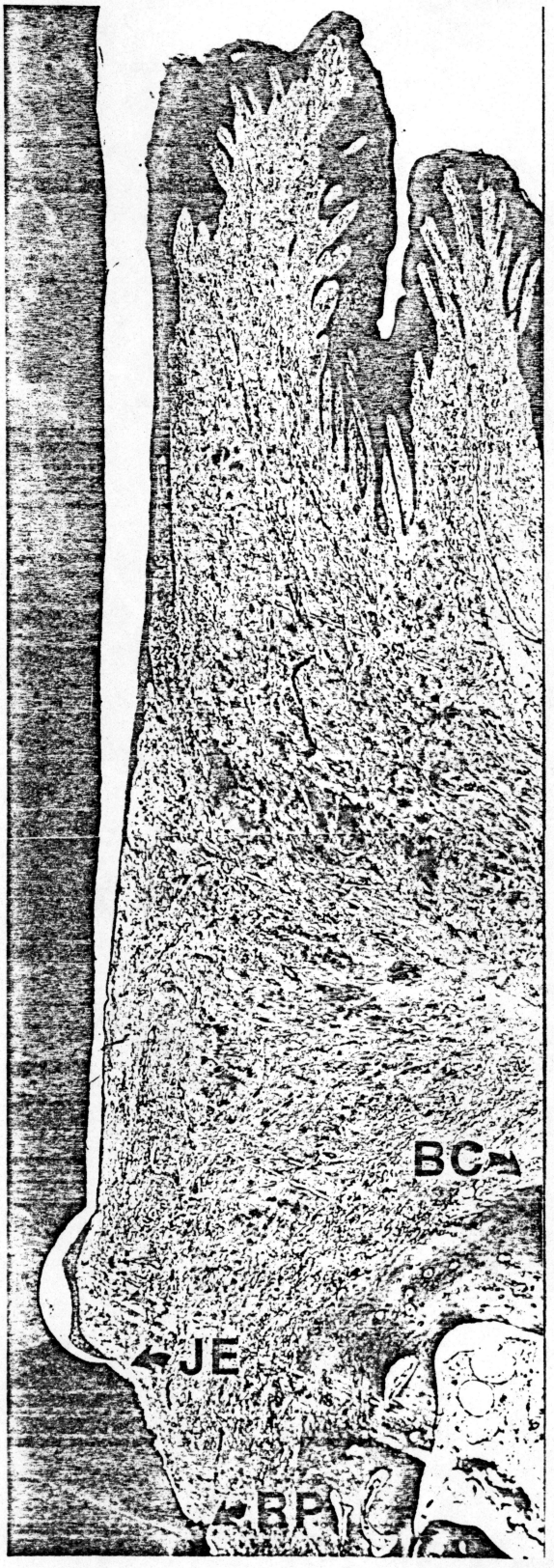
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