Cementum repair after apicoectomy in humans

J. O. ANDREASEN

Dental Department, University Hospital (Rigshospitalet), and Department of Oral Pathology, Royal Dental College, Copenhagen, Denmark

Andreasen, J. O. Cementum repair after apicoectomy in humans. *Acta Odont. Scand.* 31, 211—221, 1973.

The purpose of the present study was to study histologically the variance in cementum repair after surgical endodontic therapy. The material consisted of 35 biopsies obtained in the follow-up period after surgical treatment of periapical inflammatory changes or cystic lesions in humans. The biopsies included apex and surrounding periapical tissues. After demineralization the specimens were embedded in paraffin and serial sections were made. In a projection microscope the repair of cementum and periodontal ligaments as well as the presence of periapical inflammation and fibrous scar tissue was assessed by a histometric technique. A statistical analysis of these data revealed that the presence of functional arranged periodontal fibers was associated with deposition of a thick layer of eosinophilic cementum. Contrary in areas with scar tissue, granulation tissue or inflamed connective tissue, cementum repair was very limited and either consisted of a thin layer of basophilic cementum or no cementum deposition at all.

Key-words: Dental cementum; apicoectomy; endodontics

J. O. Andreasen, Department of Oral Pathology, Royal Dental College, Universitetsparken 4, 2100 Copenhagen Ø, Denmark

In a previous study on the repair after endodontic surgery in humans, it was found that deviations from complete regeneration of the different periodontal components often occurred (*Andreasen & Rud*, 1972). Thus the amount of cementum repair varied from no repair at all to extensive deposition of new cementum upon the resected root surface. The same applied to the periodontal ligament where some cases showed reformation of this structure whereas other cases showed replacement by fibrous scar tissue, inflamed connective tissue or granulation tissue.

The aim of the present study was to examine a possible relationship between the mode and extent of cementum repair and the type of repair of other periodontal structures after endodontic surgery in humans.

MATERIAL AND METHODS

The histologic material used in this study was part of a material previously reported consisting of 70 biopsies obtained in the follow-up period after surgical treatment of periapical inflammatory changes or cystic lesions (*Andreasen & Rud*, 1972). Out of this material 35 specimens were selected where an apicoectomy had been carried out, and where the presence of a sufficient amount of periodontal tissue allowed a histologic examination of all

Received for publication, april 17, 1973.

Table I. *Observation period before biopsy*

Observation period (years)	>1	1	2	3	4	5	6	7	8	>8
Number of cases	1	9	8	4	1	2	3	2	2	3

periodontal components. The clinical data for these biopsies including the observation periods before biopsy are listed in Tables I and II. The biopsy technique used was that described by *Nygård-Östby* (1948). With a thin surgical bur a block was removed including the apex and surrounding tissues and bone. The specimens were fixed in 10 per cent neutral buffered formalin and later demineralized in formic acid/sodium citrate. If retrograde amalgam fillings were present these were removed with a neddle or a bur after demineralization. The specimens were embedded after double infiltration in celloidin-paraffin. Nineteen specimens were cut in a mesio-distal direction along the axis of the tooth, whereas the remaining cases were sectioned in a vestibulo-lingual direction. The specimens were sectioned at a thickness of 6 to 8 μm. Sections were stained with hematoxylin-eosin and van Gieson's picrofuchsin stain (*Romeis*, 1948). Step serial sections were cut of 18 of these teeth (*Stanley*, 1957) whereas the remaining teeth were sectioned completely. An average of 41 sections was available for evaluation from each specimen. For

Table II. *Relation between the amount of cementum repair and various clinical factors*

		n	$\bar{x}$ (μm)	S.D.$_k$	Level of significance
Age at the time of operation	10—29 years	12	11.2	10.9	P>0.05[1])
	30—39 years	13	22.8	31.0	
	40—70 years	10	13.6	18.1	
Sex	Females	29	15.4	23.2	P>0.05[2])
	Males	6	16.2	15.7	
Type of tooth	Max. lateral incisors	4	24.4	24.6	P>0.05[2])
	Max. central incisors	9	23.4	35.2	
	Canines	18	8.0	10.7	
	Other locations	4	23.6	14.1	
Type of root filling	Guttapercha	27	17.0	23.4	P>0.05[2])
	Amalgam	8	11.0	12.7	
Location on the resected root surface	Vestibular-lingual	16	14.4	17.2	P>0.05[2])
	Mesio-distal	19	14.5	23.2	
Observation period	0—2 years	18	9.2	14.8	P>0.05[2])
	3—14 years	17	22.2	26.4	

[1]) One way analysis of variance according to Kruskal-Wallis
[2]) According to randomization test for two independent samples

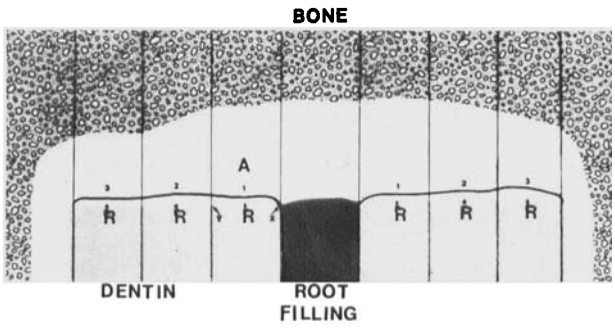

Fig. 1. Schematic drawing illustrating reference points used in the histometric analysis. The base line for the measurements is the resected root surface and the starting point the entrance of the root canal. R is the midpoint between X and Y, and at this point the thickness of newformed cementum is measured. A measuring area (A) is demarcated by the root surface and the surface of the adjacent alveolar bone and two lines perpendicular to the root surface at reference points X and Y. Registrations of cementum repair and periapical soft tissue changes are registered for the entire part of the root surface and periapical tissue (i.e. the areas denoted 1 to 3).

the histometric study the section was selected which passed through the center of the rootfilling, and where the technical quality of the section allowed evaluation of all periodontal components. A projection microscope was used for the histometric study (Reichert Visopan). Sections were projected on a 200 mm disc type screen using a magnification of 13×. A square-ruled acetate plate with 1 cm distance between the lines was placed over the projected apical area. The measuring length 1 cm on the screen accounted for 228 μm in the specimen with the magnification used. The base line for the measurements was the resected root surface and the starting point the entrance of the root canal (Fig. 1). The first measuring area comprised 228 μm of the root surface next to the rootfilling. At the midpoint between X and Y in Fig. 1, i. e. 114 μm from the rootfilling, the thickness of newformed cementum was measured with a millimeter gauge. This measurement was made perpendicular to the resected root surface using a magnification of × 50, and the measure included cementoid substance. In cases where distinction between cementoid and adjacent collagenous fibers was difficult, the line connecting the basal part of cementoblasts was considered a distinction between cementum and periodontal soft tissues. These measurements were made with a precision of 2.2 per cent (*Andreasen*, 1973).

The following variations in cementum were registered in each location of measurement according to the tinctorial properties and cellular content of cementum:

1. Eosinophilic acellular cementum.
2. Eosinophilic cellular cementum.
3. Basophilic acellular cementum.
4. Combinations of the above mentioned three cementum types.

The tinctorial properties of cementum on the resected root surface were checked with cementum on the lateral surface of the root which was in all cases of the eosinophilic type. Changes within the periapical soft tissue adjacent to the root surface was analyzed as follows. A measuring area (A) was demarcated by the root surface and the surface of the adjacent alveolar bone and two lines perpendicular to the root surface at reference points X and Y (Fig. 1). The soft tissue changes were analyzed using hematoxylin-eosin stained sections. After removal of the stain with acid alcohol the same section was stained with a van Gieson stain in order to reveal more clearly the the pre-

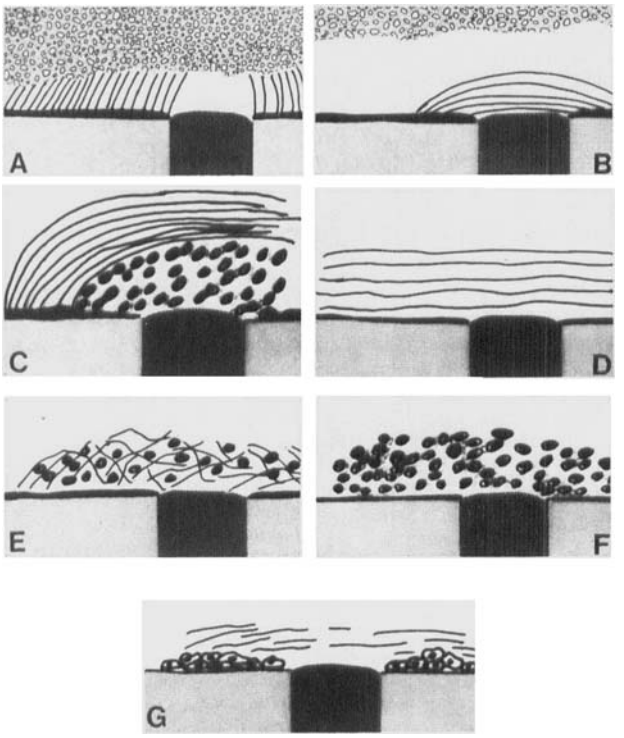

Fig. 2. Schematic drawings illustrating the classification of soft tissue adjacent to the root surface.
A, Type 1: Collagenous fibers extending from cementum to bone.
B, Type 2: Collagenous fibers extending from cementum to cementum.
C, Type 3: Collagenous fibers extending from cementum to the periphery of a granuloma or cyst.
D, Type 4: Collagenous fibers running parallel to the root surface.
E, Type 5: Connective tissue with inflammation.
F, Type 6: Granulation tissue with inflammation.
G, Type 7: Proliferation of epithelium on the root surface.

sence and arrangement of collagenous fibers. The character of the soft tissue adjacent to the cementum surface in the measuring area was then classified into the following types (Fig. 2 A to G):

Type 1. Collagenous fibers extending from cementum and inserting into alveolar bone.

Type 2. Collagenous fibers extending from cementum on one half of the root surface bridging the rootfilling and inserting into cementum at the opposite side of the root surface.

Type 3. Collagenous fibers extending from cementum to the periphery of a periapical granuloma or a cyst.

Type 4. Collagenous fibers running parallel to the root surface and without insertion into cementum (scar tissue).

Type 5. Connective tissue with acute, subacute, or chronic inflammation.

Type 6. Granulation tissue with acute, subacute, or chronic inflammation.

Type 7. Proliferating epithelium on the root surface or regular cyst formation.

Registrations of cementum repair and periapical soft tissue changes were registered for the entire part of the root surface and periapical tissue (i. e. the areas denoted 1 to 3 in Fig. 1).

Cell-countings within periodontal ligaments and scar tissue were performed using magnification of $\times$ 250 and an ocular net micrometer which delineated squares of 30 μm length in the microscope field. Countings were made in areas where collagenous fibers were sectioned parallel

Table III. *Amount of cementum repair related to type of adjacent periodontal tissue (Entire material)*

Type of periodontal repair	n	Amount of cementum repair in μm								$\bar{x}$	$S.D_k$.
		0	0.1—2.0	2.1—5.0	5.1—10.0	10.1—20.0	20.1—40.0	40.1—60.0	60.1—130.0		
Type 1. Collagenous fibers from cementum to bone	18						6	5	7	59.2	33.6
Type 2. Collagenous fibers from cementum to cementum	9			2	2	1	2		2	25.8	25.1
Type 3. Collagenous fibers from cementum to a cyst or a granuloma	4				1		1	1	1	28.2	29.1
Type 4. Collagenous scar tissue	23	12	7	2		2				1.2	2.0
Type 5. Inflamed connective tissue	18	10	2	2	3	1				2.8	4.6
Type 6. Inflamed granulation tissue	13	2	4	4	2	1				4.0	4.5
Type 7. Epithelium covering the resected root surface	16	15			1					0.4	1.4

Table IV. *Type of cementum repair related to type of adjacent periodontal tissue (Entire material)*

Type of periodontal repair	n	Type of cementum repair[2]				
		Eosinophilic acellular cementum	Eosinophilic cellular cementum	Basophilic acellular cementum	Combination of eosinophilic and basophilic cementum	No cementum repair
Type 1. Collagenous fibers from cementum to bone	18	56	44			
Type 2. Collagenous fibers from cementum to cementum	9	80	20			
Type 3. Collagenous fibers from cementum to a cyst or a granuloma	4	100				
Type 4. Collagenous scar tissue	23			38		62
Type 5. Inflamed connective tissue	18			74	3	23
Type 6. Inflamed granulation tissue	13			42	3	55
Type 7. Epithelium covering the resected root surface	16	3	3			94

[2] Calculated as the per cent of the registered measuring locations covered by the specified type of cementum

to the fiber orientation. Countings of the periodontal ligaments were confined to the lateral surface region. Squares containing bloodvessels were omitted for cell countings. A total of 200 squares was counted in each specimen.

A randomisation test and a Kruskal-Wallis one-way analysis of variance were used in the statistical analysis and a significance level of 5% was chosen as the critical probability value (Siegel, 1956).

RESULTS

In Table II the amount of cementum repair is related to various clinical factors. It appears that no one of the clinical factors was significantly related to the amount of cementum repair. The variable observation period was examined in more details in relation to the type of periodontal repair (see Table VI). In Tables III and IV the relation between the type of adjacent periodontal tissue and cementum repair is listed. It appears that

Table V. *Intra-subject relations between cementum and periodontal repair*

Type of periodontal repair			Significance level[1])	Type of periodontal repair		
Type 1 Collagenous fibers from cementum to bone				Type 2 Collagenous fibers from cementum to cementum		
	Thickness in µm				*Thickness in µm*	
n	$\bar{x}$	$S.D._k$	$P>0.01$	n	$\bar{x}$	$S.D._k$
7	66.8	15.4		7	28.40	13.8
Type 1 Collagenous fibers from cementum to bone				Type 4 Collagenous scar tissue		
	Thickness in µm				*Thickness in µm*	
n	$\bar{x}$	$S.D._k$	$0.05>P>0.025$	n	$\bar{x}$	$S.D._k$
6	53.6	11.8		6	3.2	1.25
Type 1 Collagenous fibers from cementum to bone				Type 6 Inflamed granulation tissue		
	Thickness in µm				*Thickness in µm*	
n	$\bar{x}$	$S.D._k$	$0.05>P>0.025$	n	$\bar{x}$	$S.D._k$
6	50.8	12.6		6	4.4	3.3

[1]) According to a randomisation test for matched pairs.

the presence of a functional periodontal ligament (type 1) was associated with a thick layer of eosinophilic cementum often of the cellular type (Fig. 3, A). The presence of collagenous fibers without a functional arrangement (type 2 and 3) was followed by a smaller amount of eosinophilic cementum and usually of the acellular type (Figs. 3, 4). The presence of fibrous scar tissue (Fig. 4), inflamed granulation tissue or connective tissue or epithelium (type 4 to 7) was followed by a marked decrease in the amount of cementum repair (Fig. 5, B, C). Furthermore the type of cementum repair was of the basophilic type. In many cases the resected root surface showed no cementum repair at all (Fig. 6). In order to eliminate the risk of bias due to association between the different clinical factors or external factors in the evaluation of cementum repair, the calculation shown in Table II was repeated for specimens showing various types of periodontal healing at different locations of the same tooth. In this way an intrasubject evaluation of the relation between periodontal and cementum repair was possible (Table V). It appears that the difference between cementum repair after functional collagenous fiber repair (type 1) and non-functional fiber repair (type 2) was significant. Furthermore that areas with scar tissue and inflamed granulation tissue showed significantly less cementum repair compared to areas with functional collagenous fibers (type 1) (Fig. 6, A to C).

In Table VI the relation between cementum repair and the observation period is listed. It appears that type 2 and 4 show-

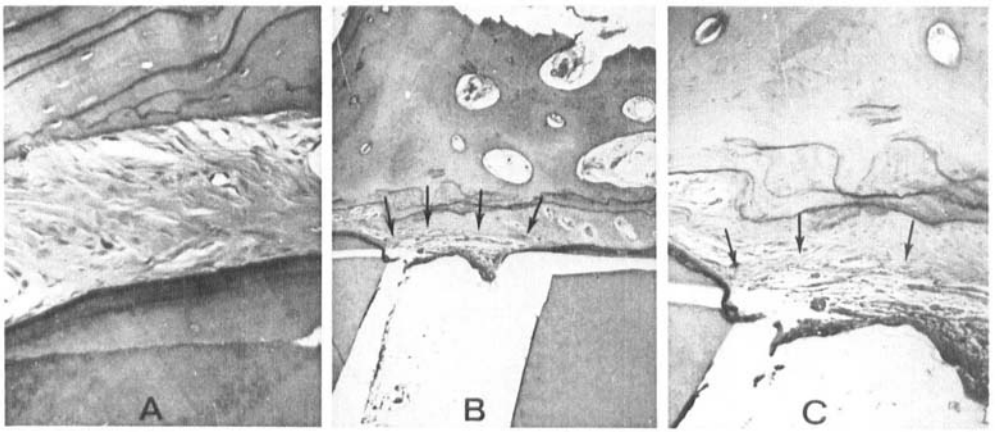

Fig. 3. *A*: Thick eosinophilic cellular layer of cementum associated with collagenous fibers extending from cementum to alveolar bone. × 156. *B*: Eosinophilic acellular layer of cementum associated with collagenous fibers extending from cementum on one half of the root surface and inserting into cementum on the opposite side of the root surface (arrows). × 25. *C*: Higher magnification of B. × 62.

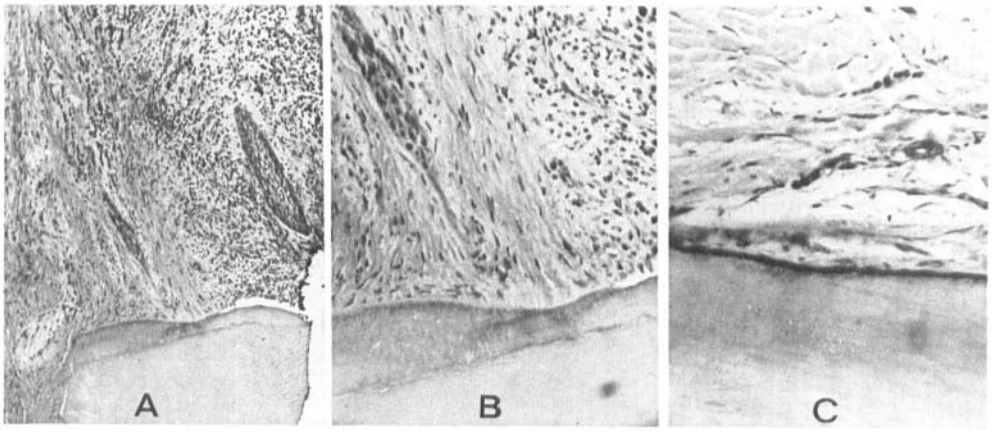

Fig. 4. *A:* Thick eosinophilic acellular layer of cementum associated with collagenous fibers extending from cementum to the periphery of a periapical granuloma. × 62. *B:* Higher magnification of A. × 156. *C:* Thin basophilic layer of acellular cementum associated with collagenous fibers running parallel to the root surface (scar tissue). × 156.

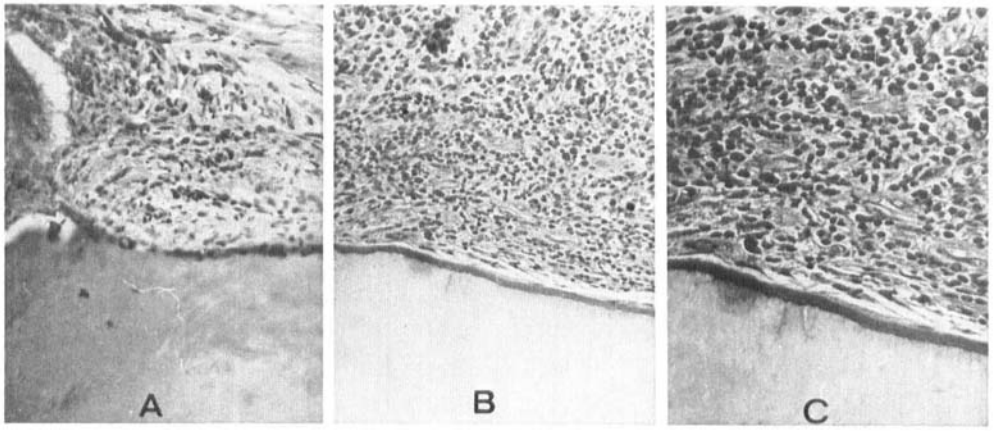

Fig. 5. *A:* Thin acellular layer of basophilic cementum associated with connective tissue with chronic inflammation. × 62. *B:* Thin acellular layer of basophilic cementum associated with granulation tissue with chronic inflammation. × 62. *C:* Higher magnification of B. × 156.

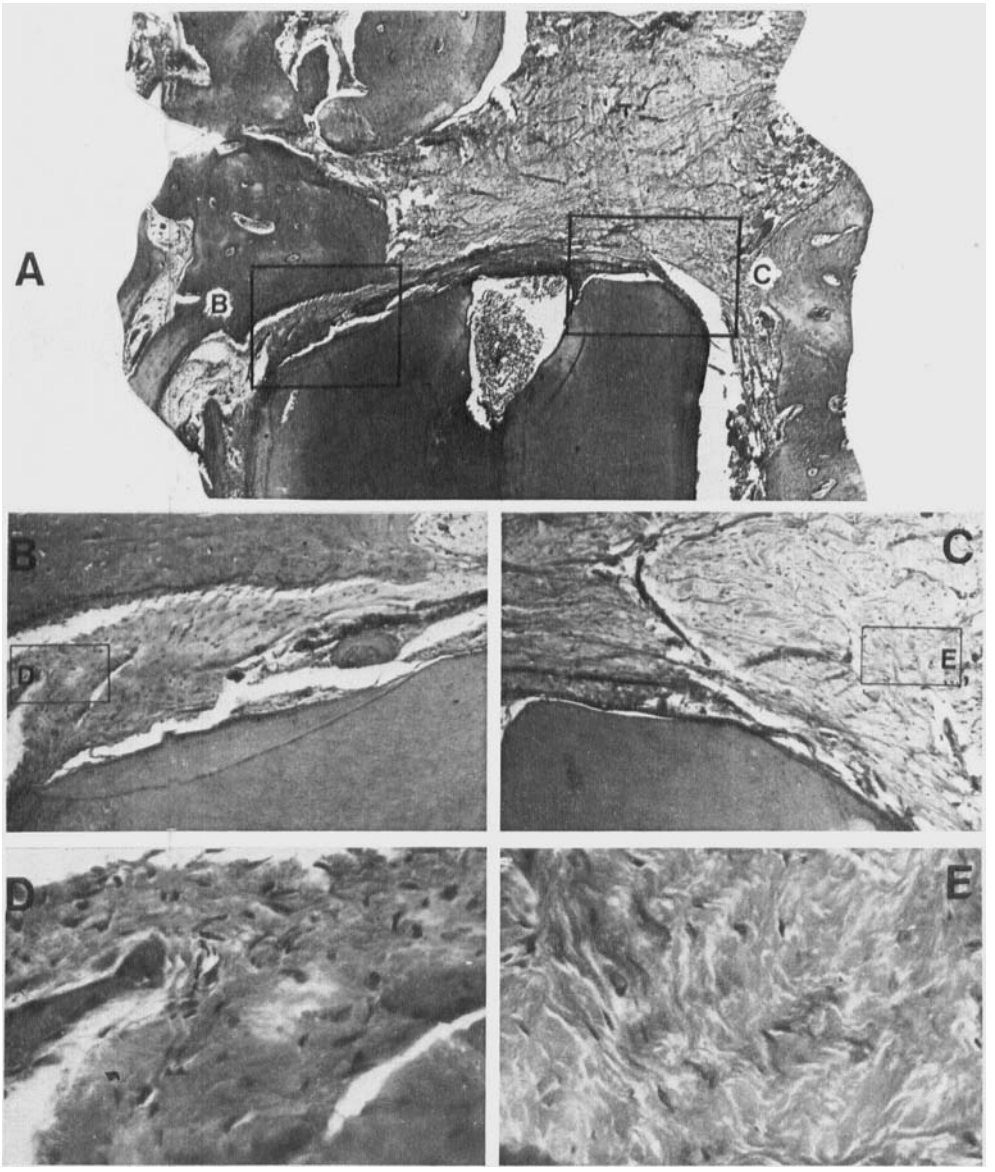

Fig. 6. Variation in cementum repair related to type of soft tissue present adjacent to the resected root surface. *A*: Low power view of surgical specimen × 25. *B*: Thick layer of eosinophilic cementum. × 62. *C*: No deposition of cementum. × 62. *D*: Periodontal ligament with a high cellular content. × 250. *E*: Fibrous scar tissue with few cells. × 250.

ed significantly more cementum repair with increasing observation periods.

In order to verify a difference in cellularity of scar tissue and periodontal ligaments, cell-countings were made within these two structures in 6 specimens (Table VII). It appears from these calculations that scar tissue contains a significantly lower amount of cells than the periodontal ligament (Fig. 6 D, E).

Table VI. *Relation between cementum repair, type of adjacent periodontal tissue, and observation period (Intrasubject analysis)*

Type of periodontal repair	Observation period and cementum repair (μm)						Level of significance[1]
	0—2 years			3—14 years			
	n	$\bar{x}$	S.D.$_k$	n	$\bar{x}$	S.D.$_k$	
Type 1 Fibers from cementum to bone	5	48.8	20.2	10	68.0	47.2	$P>0.05$
Type 2 Fibers from cementum to cementum	3	5.2	1.6	4	45.6	25.0	$0.05>P>0.01$
Type 4 Connective scar tissue	7	0.2	0.2	9	3.2	2.2	$0.01>P>0.001$
Type 6 Inflamed granulation tissue	6	0.8	1.4	7	3.6	6.2	$P>0.05$

[1] According to a randomization test for two independent samples

DISCUSSION

It appears from this study that an important association exists between cementum repair and the formation of functional periodontal fibers present. This may stress the conception that the primary function of cementum is to anchor periodontal fibers, and if no fibers are present there is consequently no use for cementum. This may explain while some teeth, even after several years, in the presence of fibrous scar tissue showed no cementum repair at all.

The type of functional arrangement of the periodontal fibers appeared to be of importance too. Thus a fiber arrangement from cementum to bone (type 1) was associated with considerably more cementum repair than fibers bridging a root filling or a granuloma or a cyst (type 2 and 3). The nearly identical cementum repair found in type 2 and 3, points towards the similar action of these fibers, namely as delimitating fibers presumably not being stressed during function. The slight amount of basophilic cementum found in the pre-

Table VII. *Number of cells per unit area in periodontal ligament and connective scar tissue (Intra-subject analysis)*

	n	$\bar{x}$	S.D.$_k$	Significance level[1]
Periodontal ligament	6	3.29	0.17	$0.05>P>0.025$
Collagenous scar tissue	6	1.18	0.28	

[1] According to a randomization test for matched pairs

sence of inflammation demonstrates that a slight cementum repair may occur even if no periodontal fibers insert in the cementum. The basophilic character of the cementum is probably a sequel to a very slow deposition rate. A similar phenomenon is found in deposition of bone where the basophilic resting lines are the expression of the deposition of bone at a very slow rate. Basophilic cementum deposits were also noted in experimental periodontal injuries in rats when an inflammation was induced during the healing process (*Andrease*, 1973).

Acknowledgment. This study was supported by a grant from the Danish State Research Foundation.

REFERENCES

Andreasen, J. O., 1973. Histometric study of healing of periodontal tissue in rats after surgical injury. Design of a standardised surgical procedure. In preparation

Andreasen, J. O. 1973. Effect of pulpal necrosis upon periodontal healing after surgical injury in rats. Int. J. Oral Surg., *2:* 62—68

Andreasen, J. O. & Rud, J., 1972. Modes of healing histologically after endodontic surgery in 70 cases. Int. J. Oral Surg., *1:* 148—160

Nygaard-Östby, B. 1948. Rotspissreseksjonens indikasjon og utførelse. Norske Tannlaegeforen. Tid., *58:* 58—87.

Romeis, B. 1948. Mikroskopische Technik. Leibnis Verlag., 15 aufl., München, p. 167

Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Company, New York, p. 88—92, 152—156, and 184—193

Stanley, H. R. 1957. The value of step-serial sectioning in the morphology study of human periodontal lesions. J. Periodont., *28:* 248—252