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BACTERIOLOGICAL STUDIES OF THE NON-VITAL PULP IN CASES WITH INTACT PULP CAVITIES

by

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The majority of the numerous studies of the bacteriology of the pulp and the periapical region have been performed on extracted teeth. Owing to the grave risk of contamination that this procedure incurs, however, it is nowdays generally the practice to take the sample from the tooth *in situ*.

Among the recent studies of the bacteriology of the nonvital pulp in which the samples have been removed *via* the root canal are those by *Grossman & Christian* (1952), *Dietz* (1952), *Slack* (1953), *Eklöf* (1955), *Cran* (1956), and *Macdonald et al.* (1957). The material of *Grossman & Christian* consisted of over 1,000 "pulpless teeth". *Grossman* (1950) defines a pulpless tooth as one the pulp of which either has been removed or is dead. *Slack* studied the bacterial flora of 514 "infected root canals". *Cran*, who compared the results of aerobic and anaerobic cultures, would appear from his earlier paper (1954) to have included vital teeth in his series. None of these authors state whether the pulp cavity was intact. In all cases samples were taken either before or after treatment.

Dietz made bacteriologic examinations of 65 pulp-involved teeth in which the pulp cavity was described as probably uncon-

taminated from the saliva. Samples were taken at the first appointment. The first culture was not performed anaerobically. Growths were obtained in the case of 19 of these 65 teeth. Direct smears from the pulp were also examined. In a few instances where there was a gangrenous odour bacteria could not be demonstrated neither by culture nor by smears.

Guthof (1953) and *Eklöf* performed studies on non-vital teeth, taking bacteria specimens before treatment was begun. Though *Eklöf* specifies whether or not the pulp cavity was open on treatment, he omits to say whether it had been opened on a previous occasion.

Macdonald & co-workers (1957) examined 46 intact teeth found to be non-vital as a result of trauma. The samples were taken under strict aseptic conditions before the pulp cavity was opened prior to treatment. The first cultures were performed only in liquid media. Growths were obtained in respect of 38 teeth, a total of 71 strains being isolated, 23 of them anaerobic. Aerobic streptococci were found in 20 cases and anaerobic grampositive cocci (anaerobic streptococci) in a further 12. A number of other strains were isolated; they were found in up to six teeth.

The reason why the present bacteriologic study was restricted to "non-vital teeth" having intact pulp cavities, although this group constitutes only a small part of a clinical endodontic material, is that, as salivary contamination was highly improbable, the path of infection might have been different from the usual one. Moreover, the group was clinically well-defined, so that the material may be expected to be homogeneous.

MATERIAL

Case material — Only single-rooted teeth with pulpal necrosis in an intact pulp cavity were accepted for the present study. Apart from those with intact crowns only teeth with superficial carious lesions, fillings and fractured enamel were included. A total of 36 such teeth were obtained over a period of three consecutive terms (1955—56) from 31 patients receiving treatment at the Department of Endodontics of the Royal School of Dentistry in Stockholm.

Nineteen of the 36 teeth had intact crowns, seventeen had superficial carious lesions, enamel fractures or minor fillings, and 28 teeth presented radiographic evidence of periapical changes. There was a gangrenous odour in one half the teeth. (Table I).

Clinical diagnosis — The pulp cavity was regarded as being intact in teeth having intact crowns, superficial lesions, fractures of the enamel or filled superficial cavities. In these cases the absence of any passage to the pulp chamber was confirmed with a fine file after carious dentine and fillings had been removed. When the pulp cavity was opened the complete necrosis of the pulp was demonstrated by exploration as far as the apex with a broach. During this examination the presence of any gangrenous odour was noted. When none could be found, and in cases of doubt, the opinions of two other persons were obtained.

METHOD

Bacteriologic technique — Strict asepsis was observed. Instruments, cellulose points, etc. were kept sterile in closed cassettes with overlapping lids.

The tooth to be examined was isolated by means of a rubber dam and then rinsed, as were the clamps and surrounding parts of the dam, with hydrogen peroxide (30 per cent) and 10 per cent iodine spirit. Immediately before the pulp chamber was opened the dentine powder from the site of drilling was transferred to dextrose serum broth and *Brewer*'s thioglycollate broth. The pulp chamber opened, samples were obtained by means of *Miller* files wrapped in cotton wool and placed on blood agar plates for aerobic and anaerobic cultures. If the pulp residue was dry, sterile physiologic saline was introduced into the chamber. Samples were taken with cellulose points for culture in dextrose serum broth and *Brewer*'s broth. Samples taken with *Miller* files wound with cotton wool were placed on two object glasses for *Gram* and *Giemsa* staining. The root canals were cleaned with root canal files and by flushing with physiologic saline. In some cases new samples were taken when the canal had been cleaned. Both the *Miller* files and the cellulose points were conducted into the periapical tissue until a pain reaction was elicited.

Table I.

Bacteriologic and clinical observations.

Case no.	Tooth (Haderup nomenclature)	Bacteriologic examination			Status of crown	Gangrenous smell	Periapical x-ray observations	Clinical symptoms		Root filled at appointment no.*	Remarks
		Direct smear		Cultivation				Before therapy	After therapy		
		Gram	Giemsa								
1	+2	+	+	+	d sil	+	+			4	
2	+1	—	+	+	intact	—	—	3	3, 6	3	Trauma (1 week)
3	1+	—	—	+	md sil	+	—	1—5	3, 6	4	
4	+1	—	—	+	m caries	+	+		6	4	
5	—1	—	—	+	intact	—	+	1, 3, 4, 5		3	
6	1+	—	—	—	intact	—	+			2	Trauma (4 months)
7	+1	—	—	—	d caries	—	—			2	Trauma (4 months)
8	—1	+	+	+	md am	+	+		4, 6	4	
9	1—	+	+	—	intact	+	+			3	Trauma (nail-biter)
10	—1	+	+	+	intact	+	+	1		3	
11	2+	—	—	—	intact	—	+	3		2	
12	+1	+	—	—	intact	—	+			2	Trauma (15 years)
13	—2	—	—	—	intact	—	+				Treatment interrupted
14	4+	+	+	+	mod am	+	—	2—5	6	6	Acute in connection with exacerbation of osteitis at —4
15	—1	+	+	+	intact	+	+			3	Trauma (5 years)
16	2 —	+	+	+	m acrylic	+	+	1, 2, 4, 5	1—7	6	Acute in connection with exacerbation of osteitis at —1
17	3+	+	+	+	d cohesive gold	+	—	1, 3, 4	1, 3, 4	4	Trauma (cohesive gold 1 month)
18	1—	+	+	—	m sil	—	+	3	1, 3, 4, 6, 7	5	
19	3—	+	+	+	intact	—	+	1—6	6	4	

20	2+	+	+	+	m sil	+	+	1—4		3	
21	+1	+	+	+	m sil	+	+	1—4		3	
22	1+	—	—	—	d sil	—	+			2	Trauma (2 months)
23	1—	—	—	—	intact	—	+			2	
24	—1	—	+	—	intact	—	+			2	Trauma (10 years)
25	2+	—	—	+	the incisal edge fractured	+	+	2, 3, 4		3	Trauma (6 months)
26	+2	—	+	+	»	+	+	2, 3, 4		3	Trauma (6 months)
27	+1	—	—	+	»	—	+			3	Trauma (6 months)
28	+2	—	—	—	intact	—	—	2		2	
29	—1	—	—	—	intact	—	+			2	
30	1+	—	—	—	intact	—	—			2	
31	+1	+	+	+	intact	+	+			3	Trauma (4 months)
32	1+	+	+	+	md acrylic	+	+	3		3	
33	1+	—	+	—	md caries	—	+	4		3	Trauma (5 years)
34	—1	+	+	+	intact	+	+	2, 3, 4	2, 3, 4	3	Trauma (4 months)
35	1—	+	+	+	intact	+	+	2, 3, 4	2, 3, 4	3	Trauma (4 months)
36	2—	—	—	—	intact	—	—	2, 3, 4	2	2	Trauma (4 months)
		17+	20+	21+		18+	28+				

No dentine specimens gave growths on culture.

LEGEND:

The figures in column *Clinical symptoms* denote:

1. pain
2. tenderness in biting
3. tenderness on palpation at apex
4. periapical tenderness
5. swelling
6. exudation
7. lymphadenitis

sil = silicate filling
am = amalgam filling
m = mesial
o = occlusal
d = distal

In the *Remarks* column the period is that elapsing after trauma.

* A further bacteriological test was taken at this appointment. In no case there was growth.

Table II.

Frequency of various cultured strains.

Alpha streptococci (viridans-lactis group)	5	
Gamma streptococci (viridans-lactis group)	1	
Str. liquefaciens	1	
Str. zymogenes	1	
Anaerobic streptococci	8	
Neisseria	2	(1 anaerobic)
Veillonella	2	
Diphtheroids	5	(4 anaerobic)
Lactobacilli	3	
Other Gram-positive rods	2	
Bacteroides	1	
Fusobacteria	11	
	42	

Mixed cultures were found in 13 cases out of 21.
Fusobacteria and streptococci were found together in 10 cases.

In the laboratory a streak of *Staphylococcus aureus* was made on the blood plates to accelerate growth and to facilitate identification of any *Haemophilus influenzae*. The anaerobic culture on blood agar was performed in an anaerobic jar, which was evacuated three times and filled with nitrogen. A vessel containing pyrogallol and potassium carbonate was placed in the jar to absorb residual oxygen.

Liquid media were incubated for one week, and aerobic and anaerobic solid media for 4 days and one week, respectively.

COMPOSITION OF THE MEDIA

Serum dextrose broth — One kilogram minced beef to one litre of water; left to stand in the cool for 24 hours; boiled for one hour. To a portion were added 20 g dibasic sodium phosphate and 10 g dextrose, and made up to one litre with the broth.

Thioglycollate broth — 3.75 ml of a 20 per cent cystine solution, 5 g dextrose, 0.75 g agar-agar, 2 g sodium chloride, 15 g Bactogen or Aminosol (protein hydrolyzate), 5 g yeast extract, 0.3 ml thioglycolic acid (conc.), 0.002 g methylene blue, pH 7.6—7.8, aqua font. ad 1,000 g.

Each tube contained 10 ml. The contents were semi-fluid. In

Table III.

Results of culture in initial medium.

No.	Initial medium				Bacteria
	Ds	Br	O	Ox	
1	+	+	+	+	1) aerobic diphtheroids
2	—	+	+	+	1) alpha streptococci
3	—	+	—	—	1) fusobacteria, 2) anaerobic streptococci, 3) anaerobic diphtheroids, 4) anaerobic Neisseria
4	+	+	+	+	1) anaerobic diphtheroids
5 a)	+	+	—	+	1) anaerobic diphtheroids
(b)	—	+	—	+)	1) fusobacteria, 2) anaerobic streptococci, 3) anaerobic diphtheroids
8 a)	—	+	—	+	1) fusobacteria, 2) Veillonella
(b)	+	+	+	+)	1) Neisseria
10 a)	—	+	—	+	1) anaerobic Gram-positive polymorphous rods
(b)	—	+	—	+)	1) Bacteroides, 2) anaerobic Gram-positive polymorphous rods
14	+	+	+	+	1) Streptococcus liquefaciens
15 a)	—	+	—	+	1) fusobacteria, 2) anaerobic Gram-positive polymorphous rods
(b)	—	—	—	—)	
16	+	+	—	+	1) gamma-streptococci (viridans-lactis group)
17	—	+	—	+	1) fusobacteria, 2) anaerobic streptococci
19	+	+	—	+	1) „ 2) alpha streptococci
20	—	+	—	—	1) „ 2) anaerobic streptococci
21	+	+	—	—	1) „ 2) „ „
25	—	+	—	—	1) „ 2) „ „
26	+	+	+	+	1) Streptococcus zymogenes
27	+	+	+	+	1) alpha streptococci
31	—	+	+	+	1) fusobacteria, 2) alpha streptococci, 3) lactobacilli
32	+	+	—	—	1) anaerobic diphtheroids
34	+	+	+	+	1) alpha streptococci, 2) anaerobic streptococci, 3) lactobacilli, 4) Veillonella
35	—	+	—	+	1) fusobacteria, 2) anaerobic streptococci, 3) lactobacilli
	12+	21+	7+	16+	

(a) = root canal sample (b) = sample from periapical area

\+ = growth

— = no growth

Ds = serum dextrose broth

Br = thioglycollate broth *(Brewer)*

O = aerobic blood agar, Ox = anaerobic blood agar

Table IV.

Comparison of clinical and bacteriologic findings.

Total	Bacteriologic findings		Clinical findings		
36	Bacteria	26	Periapical changes	22	
			No changes	4	26
			Impaired	15	
			Intact	11	26
	No bacteria	10	Periapical changes	6	
			No changes	4	10
			Impaired	2	
			Intact	8	10
Total	**Clinical findings**		**Bacteriologic findings**		
36	Gangrenous smell ..	18	Bacteria	18	
			No bacteria	0	18
	No smell	18	Bacteria	8	
			No bacteria	10	18

order to drive off the oxygen, the tubes were warmed carefully the same day as the inoculation was performed.

Blood agar — Nutrient broth (as above) with 2 per cent agar-agar, 5 per cent defibrinated horse serum.

A number of media were used for differentiation.

The differentiations were performed with the guidance of *Bergey's Manual of Determinative Bacteriology,* with certain modifications (*Frostell,* 1957).

Streptococci were differentiated according to colony morphology into alpha, beta and gamma types. Those growing as blue colonies on blood agar with gentian violet (1/20,000) were differentiated into viridans, lactis and enterococci groups. The types of enterococci were differentiated.

The lactobacilli are defined as grampositive non-sporforming rods not producing catalase and growing on tomato agar at pH 5.0 (*Charlton & Spies,* 1956). Certain lactobacilli that do not grow at such low pH (*Morris,* 1953) have been assigned to the group *atypical grampositive rods.*

Diphtheroids are corynebacteria-like grampositive rods — aerobic or anaerobic — possibly banded or beaded.

There were some grampositive rods which resembled diphtheroid ones as regards colony morphology and microscopic picture, which were inactive to carbohydrates, and which were catalase negative. These were placed in the group *atypical grampositive rods*.

Anaerobic gramnegative rods with pointed ends were classed as *fusobacteria*. Though they sometimes occurred in long filaments in cultures they were still regarded as fusobacteria if they were of typical appearance.

RESULTS

The material and results are presented in Tables I—IV.

In no case did growth occur in cultures from specimens taken from the dentine.

In the case of 21 of the 36 teeth micro-organisms were cultured. They were observed in smears from 16 of these 21 teeth and in 5 of the remaining 15 teeth. Thus, in 26 cases in all bacteria were demonstrated in either culture or smears, if not in both.

In all 21 cases in which bacteria were found in the cultures growths were obtained initially in the *Brewer* broth; on the anaerobic blood agar plate they were found in 16, in the serum-dextrose broth in 12, and on the aerobic blood agar plate in 7 cases (Table III). The types and distribution of the micro-organisms are given in Tables II—III.

Of the 26 infected teeth 22 presented radiographic evidence of periapical destruction, while the 10 uninfected teeth presented such signs in 6 cases.

Eleven of the 26 infected teeth had intact crowns, while 8 of the 10 uninfected teeth were intact.

In 17 of the 18 teeth with gangrenous odour micro-organisms were obtained in the cultures, and in the remaining tooth they were observed in the smear. In the 18 teeth with no gangrenous odour, cultures demonstrated micro-organisms in 4 cases and the smears in a further 4.

DISCUSSION

The smallness of the material is due to the strictness of the clinical selection made. Thus, no teeth with crown restorations were included, as in such cases it is impossible to decide whether the pulp cavity is intact unless the crown is removed. Many teeth with fillings had to be excluded because communication could be demonstrated between cavity and pulp chamber. The material contained only singlerooted teeth, chosen because it is easier to take bacterial samples from the anterior part of the dentition.

The specimens were taken through the root canal with the tooth *in situ*. This procedure, which is a simple one to perform, involves less risk of contamination than when samples are taken in connection with apical resection or after extraction.

In the present study the risk of contamination existing when samples are taken from teeth having deep proximal cavities was avoided by selecting teeth with essentially intact crowns, and entry to the pulp chamber could thus be made at a distance from the edge of the rubber dam and the gingival pocket.

The samples were taken on the occasion when the pulp cavity was first opened, before any form of treatment had been performed. In many studies no distinction was drawn between such samples and those taken after one or more stages of treatment (for instance, *Grossman, Slack, Cran*). This gives a false picture of the original bacterial flora, since some micro-organisms are particularly sensitive to treatment, while others are more resistent. Thus, *Eklöf* found that anaerobes rapidly disappeared during the course of treatment. Enterococci and yeasts were, on the other hand, particularly resistent (*Bender & Seltzer*, 1950, 1952) — for instance, to local antibiotic therapy.

It was impossible to demonstrate any definite connection between the infection in the pulp cavity and periapical resorptive osteitis. Such changes occurred in this material, as in others, in teeth with and without manifest infection. However, negative cultures and smears are insufficient evidence that an autolysis of pulp would give rise to apical changes visible in the radiographs.

In all cases with gangrenous odour bacteria were found. The presence of a gangrenous odour when the pulp cavity is first

opened could therefore be accepted as proof of infection. *Dietz* reports several cases with gangrenous odour in which micro-organisms could neither be demonstrated by culture nor in smears. It should be borne in mind, however, that the sense of smell is highly subjective. Moreover, odour cannot be used as a criterium of infection after treatment has been proceeded with. There seems to be no study of the bacteriology of pulpal gangrene in which the relationship between the gangrenous odour and the occurrence of particular micro-organisms has been examined. The anaerobic bacteria are probably a factor of significance in this connection. *Bøe* (1941) considered that a very pronounced putrescent odour is present where fusobacteria and streptococci grow together. In the present study it was observed that a strong odour was generally associated with anaerobically incubated blood agar plates that had been inoculated with samples from infected root canals, whereas in the case of aerobically incubated plates the odour was much weaker.

The study reveals the importance of using anaerobic as well as aerobic media in studies of the root canal flora, since the pulp cavity seems to provide an environment poor in oxygen, especially in the case material in question.

The samples were transferred at the chair to both fluid and solid media. The use of solid media permits an estimate to be made of the degree of infection, i.e. the number of bacteria in the sample and the quantitative composition of the flora. It was occasionally possible to exclude one or another colony of, say, *Staphylococcus albus,* that lay outside the inoculation smear. It sometimes happens in laboratory work that only one particular strain — say *Staphylococcus albus* — is demonstrated in fluid media whereas in solid media it is absent, and a more typical root canal flora can be shown.

As previously shown, micro-organisms were observed in direct smears from the pulp chamber in 5 cases, although there was no growth in the cultures. It is possible that the micro-organisms observed in the smears were dead ones, and this would then indicate only that there had been infection previously. It is more probable, however, that the culture technique was not suitable for these micro-organisms. So long as the substrates and methods used clearly do not provide suitable conditions for the complete

root canal flora, it will be necessary — in studies of this type, at least — to examine stained smears from the pulp cavity. Anaerobic culture in hydrogen might have provided better results.

Practically all studies of root canal bacteriology have, however, been performed with liquid media at the first isolation in which certain types are overgrown by others so as to be unidentifiable.

The results of cultures on dentine drillings from the cavity walls and the site of exposure suggest that the infection had probably not reached the pulp chamber from the oral cavity *via* the areas from which dentine was removed for culture. Other paths of infection — for instance, blood and lymph vessels — must be considered. In spite of this, the flora was generally such as is commonly found at sites in the oral cavity where there is little oxygen.

Growths were obtained in the present study in 21 of 36 teeth, and bacteria were demonstrated in the smears of a further 5. *Dietz* (1952) obtained growths in 19 out of 65 cases, the smears revealing micro-organisms in a further 16 cases. He considered that the bacteria in these teeth were not vital. A more likely explanation is that the micro-organisms could not multiply because the samples were not cultured anaerobically from the start, or because for other reasons the media provided an unfavourable environment.

The study performed by *Macdonald et al.* (1957) bears a close resemblance to the present one. The frequencies of occurrence of streptococci for aerobic growth were 52 and 38 per cent respectively, and for anaerobic conditions 31 and 38 per cent. In both materials there was a number of other aerobic and anaerobic strains. The most striking difference in the results is the frequent occurrence of fusobacteria in the present material — 52 per cent. Recently, *Shovelton & Sideway* (1960) have shown that anaerobes are very common in pulpless teeth. They found fusobacteria in nearly 30 per cent of their positive cultures.

Non-infected pulpal necrosis is certainly not so common as the literature would suggest. From the clinical standpoint they should, however, be revealed by the first bacteria samples so that the appropriate medication can be given. In cases where the

initial bacteria samples gave no culture the treatment could generally be completed at two appointments.

In one half of the cases in which bacteria were demonstrated there was a pure culture. The mixed cultures disclosed a strikingly high frequency of fusobacteria. It is remarkable that the combination of streptococci and fusobacteria is so common. *Bøe* (1941) supposed that the presence of the former favoured the growth of the latter.

No study was made of whether the isolated micro-organisms were obligate anaerobes or whether they were facultative anaerobes, which did not grow under aerobic conditions in the first culture. However, it proved that certain strains that grew only under primarily anaerobic conditions on the first isolation appeared after some time in an aerobic environment. In Case 21 the growth appeared in the *Brewer* tube after 48 hours, but in the dextrose tube it did not appear for 6 days.

SUMMARY

The authors have performed a bacteriological investigation on 36 teeth with non-vital pulps, the pulp cavities of which were intact, i.e. the pulp cavity was surrounded by hard dentin. Only teeth with intact crowns or teeth with enamel fractures or small cavities were studied. The samples were cultured aerobically and anaerobically on solid and liquid media. Smears were taken from the pulp debris for microscopical examination. All the samples were taken from the root canals, when the pulp chambers were opened for the first time.

Results

1. Micro-organisms were found in 26 teeth, 22 of which had roentgenologically visible changes around their apices. Six out of the remaining 10 non-infected teeth had such changes.

2. Eleven out of the 26 infected teeth and 8 out of the 10 non-infected teeth had intact crowns.

3. In one half of the cases (18 teeth) there was a gangraenous odour. The presence of micro-organisms was demonstrated in smears or by culture methods in all these cases.

4. In the other half of the material (18 teeth) micro-organisms were found in 8 teeth only.

5. Micro-organisms were cultivated from 21 out of the 36 teeth. In 5 further cases micro-organisms were found in the smears.

6. In all instances when growth was obtained in the media (21) there was growth in *Brewer*'s thioglycollate medium. In 16 out of these cases growth was present on anaerobically incubated blood agar as well. In 12 out of the 21 cases there was growth in the serum-dextrose liquid medium, and in 7 instances on aerobically incubated blood agar.

7. Most of the strains isolated were anaerobic. A mixed culture consisting of Fusobacterium and streptococci sometimes together with other strains, was found in 10 teeth. Tables II and III.

In the discussion the authors claim that the literature on the bacteriology of the non-vital pulp is partly misleading since the clinical grouping of the material is often neglected. In many studies the sampling has been performed after or during root canal treatment, which must have affected the results. The importance of a suitable anaerobic technique is stressed.

RÉSUMÉ

ÉTUDE BACTÉRIOLOGIQUE DE LA PULPE NON VITALE DANS DES CAS A CHAMBRES PULPAIRES INTACTES

Les auteurs ont effectué une étude bactériologique de 36 dents a pulpe non vitale dont la chambre pulpaire était intacte, c'est-à-dire dont la pulpe était entourée de dentine dure. Seules des dents ayant des couronnes intactes, ou des dents présentant des fractures de l'émail ou de petites cavités ont donc été étudiées. Les prélèvements ont fait l'objet de cultures aérobies et anaérobies sur milieux solides et liquides. Des frottis pour examen microcospique ont été obtenus avec des débris pulpaires. Tous les prélèvements ont été faits dans le canal radiculaire, lors de la première ouverture de la chambre pulpaire.

Résultats

1. Des micro-organismes ont été trouvés dans 26 dents, dont 22 présentaient autour des apex des changements visibles à la radiographie. Parmi les 10 dents non infectées restant, 6 dents présentaient de tels changements.

2. 11 des 26 dents infectées et 8 des 10 dents non infectées avaient des couronnes intactes.

3. Dans la moitié des cas (18 dents), il y avait une odeur gangréneuse. La présence de micro-organismes dans tous ces cas a été démontrée par frottis ou par culture.

4. Dans l'autre moitié des cas (18 dents), on n'a trouvé de micro-organismes que dans 8 dents.

5. Des micro-organismes ont été cultivés à partir de 21 des 36 dents. Dans 5 autres cas on a trouvé les micro-organismes dans les frottis.

6. Dans tous les cas où l'on a obtenu un développement sur milieu (21), il se produisait un développement sur milieu au thioglycollate de *Brewer*. Dans 16 de ces cas, le développement se faisant aussi sur gélose au sang en incubation anaérobie. Dans 12 des 21 cas, le développement se faisait sur milieu liquide sérum-dextrose, et dans 7 exemples sur gélose au sang en incubation aérobie.

7. La plupart des espèces isolées étaient anaérobies. Une culture mixte composée de Fusobacterium et des streptocoques a été trouvée dans 10 dents, parfois avec d'autres espèces. Tableaux II et III.

Dans la discussion, les auteurs déclarent que la litterature sur la bactériologie de la pulpe non vitale peut en partie induire en erreur, puisque le classement clinique des specimen est souvent négligé. Dans bien des études, les prélevements ont été faits après ou pendant un traitement de canaux, ce qui doit avoir influé sur les résultats. L'importance d'une technique anaérobie convenable est soulignée.

ZUSAMMENFASSUNG

BAKTERIOLOGISCHE UNTERSUCHUNGEN DER NICHT VITALEN PULPA IN FÄLLEN MIT INTAKTEN PULPARÄUMEN

Die Verfasser haben von harter Dentinschicht umgebene Pulparäume mit avitaler Pulpa bei 36 Zähnen bakteriologisch untersucht. Das untersuchte Material besteht also nur aus Zähnen mit intakten Kronen und Kronen mit oberflächlichen kariösen Schäden, Schmelzfrakturen oder Füllungen. Die Untersuchung um-

fasst einerseits Studium von Ausstrichpräparaten des Pulpainhaltes, andererseits aerobe und anaerobe Züchtung auf festem und flüssigem Substrat. Die Entnahme der Proben erfolgte via Wurzelkanal beim ersten Öffnen des Pulparaumes.

1. Bei 26 Zähnen konnten Mikroorganismen nachgewiesen werden. Bei 22 dieser Zähne wurden röntgenologisch periapikale Veränderungen beobachtet. Von den restlichen 10 Zähnen des Untersuchungsmateriales, bei denen kein Vorkommen von Mikroorganismen bewiesen werden konnte, hatten 6 Zähne röntgenologisch nachweisbare periapikale Veränderungen.

2. In der genannten Gruppe von 26 Zähnen befanden sich 11 Zähne mit intakten Kronen, während 8 Zähne der restlichen 10-Zähnegruppe voll intakt waren.

3. In der Hälfte der Fälle (18 Zähne) konnte Gangrängeruch festgestellt werden. Mittels Züchtung oder Ausstrich wurden hier Mikroorganismen nachgewiesen. Bei der anderen Hälfte wurden in 8 Fällen Mikroorganismen entdeckt.

4. Bei 21 der 36 Zähne wurden Mikroorganismen durch Züchtung nachgewiesen, in weiteren 5 Fällen wurden Bakterien im Ausstrich beobachtet.

5. In allen Fällen (21), in denen Wachstum durch Züchtung beobachtet wurde, wurde das Wachstum im Thiokollatrohr erreicht. In 16 Fällen kam es zu Wachstum auf anaerobem Blutagar, in 12 Fällen im Serum-Dextroserohr und in 7 Fällen auf der aeroben Blutagarplatte.

6. In keinem der untersuchten Fälle konnten Mikroorganismen im Dentinstaub der Trepanationsstelle, in dem von den harten pulpalen Dentinwänden entfernten Material, unter Füllungen oder im kariösen Defekt nachgewiesen werden.

7. Die meisten isolierten Stämme waren anaerob. In 10 Zähnen wurde eine, aus Fusobakterien und Streptokokken, eventuell zusammen mit anderen Arten bestehende Mischflora angetroffen. Tabelle II und III.

In der Diskussion wird hervorgehoben, dass das Schrifttum teilweise irreführend ist, da in den meisten Arbeiten die klinische Gruppierung des Materiales unvollständig und in vielen Fällen die Entnahme der Gewebsproben nach einer Behandlung vorgenommen worden ist. Die Bedeutung einer guten anaeroben Züchtungstechnik wird unterstrichen.

REFERENCES

Bender, I. B. & S. Seltzer, 1950: Treatment of penicillin-streptomycin resistent organisms in the root canals of infected pulpless teeth. Oral. Surg. *3:* 802.

—»— 1950: The use of penicillin-streptomycin in the treatment of infected pulpless teeth. Oral. Surg. *3:* 1070.

—»— 1952: Combination of antibiotics and fungicides used in the treatment of the infected pulpless tooth. J. Amer. dent. Ass. *45:* 293.

Bergey's Manual of Determinative Bacteriology, 1948.

Bøe, J., 1941: Fusobacterium. Det norske Videnskaps-Akademi i Oslo. I. Math. Naturv. Klasse, no 9.

Charlton, G. & H. G. Spies, 1956: The relative efficiency of two selective media for the isolation of lactobacillus species from the mouth. J. dent. Res. *35:* 800.

Cran, J A., 1954: Study of the pathology and bacteriology of the pulpless tooth and its bearing on treatment. Aust. J. Dent. *58:* 291.

—»— 1956: Study of the pathology and bacteriology of the pulpless tooth and its bearing on treatment. Aust. dent. J. *1:* 161.

Dietz, V. H., 1952: Intracutaneous tests using filtrates prepared from pathologic pulps of human teeth, with special reference to rhematoid arthritis. Oral. Surg. *5:* 315, 418.

Eklöf, D., 1955: Bakteriologisk kontroll vid rotbehandling och rotspetsresektion. Svensk tandläk. Tidskr. *48:* 195.

Frostell, G., 1957: Bakteriologisk diagnostik vid Tandläkarhögskolan i Stockholm. Sverig. Tandläk.-förb. Tidn. *49,* 229.

Grossman, L. I., 1950: Root Canal Therapy: Lea & Feabiger, Philadelphia.

Grossman, L. I. & C. K. Christian, 1952: End-point study of bactericidal effect of antibiotics used in endodontics. J. dent. Res. *31:* 42.

Guthof, O., 1953: Zahnbakteriologische Fragen in Zusammenhang mit Wurzelbehandlung und Herdinfektion. Zahnärztl. Rdsch. *62:* 190.

Macdonald, J. B., G. C. Hare & A. W. S. Wood, 1957: The bacteriologic status of the pulp chambers in intact teeth found to be nonvital following trauma. Oral. Surg. *10:* 318.

Morris, E. D., 1953: The bacteriology of the oral cavity. Brit. dent. J. *95:* 259.

Shovelton, D. S., & D. A. Sideway, 1960: Infection in root canals. Brit. Dent. J. *108:* 115.

Slack, G. L., 1953: The bacteriology of infected root canals and *in vitro* penicillin sensitivity. Brit. dent. J. *94:* 211.

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