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BACTERIOLOGICAL SAMPLING FROM ROOT CANALS DIRECTLY AFTER CHEMOMECHANICAL TREATMENT

A CLINICAL AND BACTERIOLOGICAL STUDY

by

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INTRODUCTION

The primary purpose of conservative root canal therapy involving infected pulpless teeth is the elimination of the infection before permanent obturation of the canals. Usually mechanical instrumentation and irrigation with antibacterial solutions in combination with an antimicrobial dressing are sufficient to reach this goal. The effect achieved by the methods mentioned above can usually be checked at the second appointment by making a microbiological examination.

The subject of many investigations has been to find the antibacterial method having the greatest effect. *Strindberg* (1965) reported that the mechanical preparation and irrigation of the root canals with antibacterial solutions alone produced a high percentage of non-infected root canals at the second appointment. In this study he also compared the results of earlier investigations. They can be divided into three main groups with respect to the antibacterial methods used:

A. mechanical preparation only.
B. mechanical preparation with antiseptic treatment.
C. mechanical preparation with antiseptic treatment and antibacterial dressing between treatments.

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The distribution of negative cultures obtained after treatment in the groups mentioned above was compiled by *Strindberg* (1965) from the results of several authors, *(Grossman & Stewart*, 1949; *Ostrander & Crowley*, 1948; *Soul*, 1953; *Auerbach*, 1953; *Rottke*, 1954; *Stewart*, 1955; *Söndergaard*, 1956; *Breed & Smith*, 1957; *Cran*, 1957; *Ingle & Zeldow*, 1958a; 1958b; *Lichtenberg Crone*, 1960; *Melville & Slack*, 1961; *Stewart, Cobe & Rappaport*, 1961; *Engström & Frostell*, 1962; *Nicholls*, 1962; *Stewart & Gautieri*, 1962; *Grahnén & Krasse*, 1963; *Strindberg*, 1963; *Zeldow & Ingle*, 1963; *Grossman*, 1965; *Strindberg*, 1965;). The table has been somewhat modified by the present author (Table I). In the respective groups the mean values were based on investigations comprising different number of cases and that fact has not been statistically considered.

Table I.

Percentile distribution of negative cultures obtained after (A) mechanical preparation only, (B) mechanical preparation with antiseptic treatment, (C) mechanical preparation with antiseptic treatment and antibacterial dressing between treatmentsc.

	A	B	C
Negative cultures after first treatment	23	66	
Negative cultures at the second appointment (before treatment)	27	65	70

Two interesting conclusions can be drawn from the above table:

1. The antibacterial dressing seems to have had a poor effect.
2. The correspondence between cultures taken after the first and before the second treatment was high.

The last conclusion must be drawn with great reservation. The reason is that many factors may influence the results of the microbiological examination. Such factors are frequency of initially infected teeth, inactivating agents used, sampling techniques, cultivation methods, leakage and contamination.

The object of the present study was to investigate the possibility of reducing the number of treatments in conservative root canal therapy by studying the reliability of microbiological samples from the apical region of the root canal taken immediately after antibacterial treatment and use of an inactivating agent. Thus a comparison was made between the growth frequency of samples taken after the first treatment with that of samples taken at the second visit before treatment. To evaluate the risk of leakage and contamination which could not be detected clinically, the microbial flora found in positive cultures was studied with respect to its type.

MATERIAL AND METHODS

The material consisted of all those teeth which had received conservative root canal therapy by the author and an experienced specialist over a period of one year. Of the 222 teeth thus treated, 15 teeth were excluded due to leakage of the temporary filling site resulting in a contamination of the root canal by oral microorganisms. The case material therefore consisted of 207 teeth in 190 patients.

At the first appointment the pulp chamber was prepared under aseptic conditions. Sampling fluid VMG I (*Möller*, 1966) was instilled into the root canals which were rinsed until an initial sample could be taken from the apical part of the root canal. The samples were taken according to the so called PMR-method, implying pumping with a file and removal of all fluid from the canal with charcoal paper points. The paper points were transferred to a liquid medium for immediate incubation.

For the primary cultivation a liquid medium was used containing Brewer's thioglycollate broth to which was added 10 per cent inactivated horse serum and 10 per cent gelatin. Incubation at 37°C was carried out until growth was registered or up to 10 days. Further cultivation and identification of microorganisms was made on liquid and solid media, according to Bergey's Manual of Determinative Bacteriology as modified by *Engström & Frostell*, (1957).

The antibacterial treatment was started by instillation of a small amount of hydrogen peroxide 30 per cent in the canals. An excess of sodium hypochlorite 0.5 or 1.0 per cent was then introduced by a syringe. In 140 teeth the treatment was carried out with a 0.5 per cent solution, and in 67 teeth with 1.0 per cent solution. In other respects the conditions were indentical in the investigations.

The continued rinsing and mechanical preparation of the canals was carried out by alternate irrigation and suction, thus removing the necrotic remnants and dentine chips from the canals.

After the antibacterial treatment was completed, the canals were dried and sterilization of the field of operation with 10 per cent iodine tincture was repeated. The pulp chamber was filled with 5 per cent sodium thiosulfate solution which was pumped into the canals for one minute with paper points. Sodium thiosulfate acts as an inactivating agent against chlorine- and iodine-ions. The canals were then dried and a sample was taken in accordance with the method described. This direct sample was called Di-sample.

Finally sterile unprepared paper points, containing *no* antibacterial agent, were enclosed in the canals and the cavity was sealed with first a cotton pellet and then zinc oxide-eugenol cement.

At the second appointment, after an average time of 7 days, the enclosed points were taken out for cultivation (S-sample). The canals were then filled with sampling fluid VMG I and a sample was taken again in accordance with the PMR-method (A-sample). After this, the canals were rinsed and cleaned and an antibacterial dressing was enclosed in the pulp cavity. The conservative treatment was continued until negative samples were obtained. The canals were then filled with guttapercha and chloroform resin in the ordinary way.

All in all, four bacteriological samples were taken from each tooth. 1) I-sample before the antimicrobial treatment intended to show initial infection; 2) Di-sample directly after the preparation and antimicrobial treatment of the canal; 3) and 4) S- and A-samples at the second appointment.

RESULTS

The distribution of the teeth according to the number of roots is shown in Table II. As seen, single rooted teeth were represented in approximately half of the cases.

The distribution of the material according to upper and lower jaw was rather even (Table III). The pulpal and periradicular conditions are further shown in Table IV.

Table II

Distribution of teeth according to the number of roots

	Number of teeth	%
Single-rooted teeth	106	51.2
Two-rooted teeth	37	17.9
Three-rooted teeth	64	30.9
Total:	207	300

Table III

Distribution of teeth according to upper and lower jaw

	Number of teeth	%
Upper jaw	111	53.6
Lower jaw	96	46.4
Total:	207	100

Out of 207 treated cases 84.5 per cent showed agreement between the bacteriological sample taken after the first treatment (Di-sample) and at the second visit (S-sample or A-sample). (Table V). Among these cases two types of agreement could be noticed; one group in which negative Di-samples at the first visit could be verified in samples at the second visit (54.6 per

Table IV

Distribution of teeth according to pulpal and periradicular status

	Osteitis < 3 (mm)	Osteitis > 3 (mm)	Periapical cyst	Normal periap. contour	Number	Per cent
Pulpal necrosis	55	37	4	18	114	55.1
Pulpal gangrene	10	8	0	3	21	10.1
Previous amputation	9	1	0	7	17	8.2
Previous root-filling	23	16	2	14	55	26.6
Total number	97	62	6	42	207	
Per cent	46.9	29.9	2.9	20.3		100.0

Table V

Distribution of diagnosis in the alternative series of samples

	Alternatives	Samples I	Di	S	A	Osteitis < 3 (mm)	Osteitis > 3 (mm)	juxta radicular	fistula	Normal Periapical cyst	Normal periap. contour	Number	Percent
Agreement between Di & S/A	1	—	—	—	—	19	3			5	22	49	23.7
	2	+	—	—	—	32	19	7	6	1	12	64	30.9
	3	+	+	+	+	20	27	7	2		2	49	23.7
	4	+	+	+	—		1					1	0.5
	5	+	+	—	+	6	4		1			10	4.8
	6	—	+	+	+		2					2	1.0
Total						77	56			6	36	175	84.6
Disagreement between Di & S/A	7	+	+	—	—	14	2	3	2		3	19	9.2
	8	—	+	—	—	3	1				1	5	2.4
	9	+	—	+	+	1	1	1	1		1	3	1.4
	10	+	—	—	+	1	2	2	2			3	1.4
	11	—	—	—	+	1					1	2	1.0
Total						20	6			0	6	32	15.4
Total number						97	62			6	42	207	100

Table VI

Distribution of cases with negative I-samples according to diagnosis

	Osteitis					
	< 3 (mm)	> 3 (mm)	Periapical cyst	Normal periap. contour	Number	Per cent
Pulpal necrosis	10	3	2	11	26	45
Pulpal gangrene	0	1	0	0	1	2
Previous amputation	3	0	1	3	7	12
Previous root-filling	10	2	2	10	24	41
Total number	23	6	5	24	58	
Per cent	40	10	9	41		100

cent), and one group where persisting infection was found in the Di-sample and at the second visit. (29.9 per cent of cases).

Out of the total material 15.5 per cent showed disagreement. Two main types of discrepancy could be distinguished here. In one group (11.6 per cent) bacterial growth, registered in samples from the pulp cavity after debridement, could not be verified at the second visit. In the other group the samples taken after debridement showed no bacterial growth but the bacteriological samples taken at the second visit showed growth (3.8 per cent).

Negative Di-samples were found in 58.4 per cent of all cases and at the second visit negative A-samples were found in 66.2 per cent. In one case only was the S-sample positive when the A-sample was negative.

Fifty-eight teeth showed no growth of bacteria in the initial samples (28.0 per cent). In this group x-ray examinations revealed a relatively low frequency of periapical lesions greater than three mm. in diameter. (Table VI). A rather high fre quency of teeth with previously filled root canals and teeth with necrosis (without gangrene) can also be noted. 7 out of the 58 cases showed positive Di-samples. Of these, 3 cases had been treated previously by another dentist and had already received an antibacterial dressing.

The increase in the concentration of the hypochlorite solution from 0.5 per cent to 1.0 per cent in the later part of the experiment did not significantly influence the relative frequency of bacterial growth.

The frequency of different types of microorganisms in the samples is given in Table VII.

Exacerbation between treatments was noticed in 9 cases (4 per cent), in 7 cases there was pain and swelling, in 2 cases tenderness only.

Table VII

Microorganisms isolated in the different positive samples. Number of strains

Main microbial group	Microorganisms isolated (genus or group of genus)	Samples I	Di	S	A
Streptococci, Lactobacilli (facultatively anaerobic)	Streptococcus »The viridans group» (α)	60	28	23	27
	Streptococcus »The viridans group» (γ)	41	14	11	13
	Streptococcus »The enterococcus group»	26	12	13	13
	Lactobacillus	52	21	13	15
	Total	179	75	60	68
	Per cent of positive samples	57.7	47.2	65.2	62.4
Anaerobic bacteria, (strictly nonsporulating)	Peptococcus, Peptostreptococcus	25	17	8	9
	Veillonella	24	9	5	7
	Bacteroides	3	0	0	1
	Fusobacterium	8	5	3	6
	Corynebacterium	6	4	4	3
	Eubacterium	28	16	8	9
	Actinomyces	2	1	0	0
	Leptotrichia	4	3	2	2
	Total	100	55	30	37
	Per cent of positive samples	32.3	34.6	32.6	33.9
»Other» microorganisms	Micrococcus	13	9	1	1
	Staphylococcus epidermidis	3	5	0	0
	Gaffkya and Sarcina	0	0	0	0
	Neisseria	0	0	0	0
	Corynebacterium	9	11	0	1
	Pseudomonas, Proteus, Escherichia	5	3	1	1
	Candida	1	1	0	1
	Total	31	29	2	4
	Per cent of positive samples	10.0	18.2	2.2	3.7
	Total number	310	159	92	109
	Per cent	100	100	100	100

DISCUSSION

If the period of time between the antibacterial treatment and the sampling is short, or if the duration of the antibacterial effect of the medicament is long, it will be necessary to eliminate the agent by treatment with an antidote.

The results show that a bacteriological sample taken after instrumentation of a root canal and irrigation with sodium hypochlorite can be trusted with confidence, provided that inactivation of the antibacterial agent with sodium thiosulphate has been carried out. Thus, in all, only 3.8 per cent of the teeth gave false negative cultures.

The fact that the Di-samples were negative in 58 per cent, and the S- or A- samples in 66 per cent, was due to 24 cases which yielded positive cultures at the first visit and turned negative at the second visit. One reason for this may be that contamination occurred when the Di-samples were taken and thus false positive cultures were obtained. The composition of the flora in these teeth makes it probable that contamination had occurred in 2 cases, but a correction of the figures did not influence the numerical results to such an extent that the conclusions were influenced and has therefore not been given. Another reason for the higher frequency of negative samples at the second visit may be that remaining antibacterially influenced organisms had not been able to survive under the changed conditions in the root canal. The size of the periapical changes were in all these cases almost always less than three mm. (Table V). Two of the cases had fistulas which were closed at the second visit.

One would expect that the greatest risk in taking samples directly after debridement would be to obtain false negative cultures owing to remaining effects of the antibacterial agents in the root canal. In Table V it is shown that there is a disagreement in the alternatives 9, 10 and 11 between the Di-samples which were negative, and the S- or A-samples which were positive. All in all, this reason for disagreement was found in 8 teeth, of which 3 were molars and 5 were incisors. For 3 out of these 5 incisors it was found that juxtaradicular destructions with fistulas remained open at the second visit. Anaerobic bacteria from the oral cavity were dominant among the microorganisms in these samples, which makes an invasion of microorganisms through the fistula between visits a possible explanation.

Another type of disagreement, and the most common as mentioned above, was that a positive Di-sample was followed by negative cultures at the second visit. In most similar earlier experiments, the tendency has been reversed, i.e. a higher relative frequency of positive cultures has been more common at the second visit (Table I, group B). One reason for the discrepancy may be that in the earlier investigations inactivators of antibacterial agents have not been used, resulting perhaps in false negative cultures after debridement.

The risk of leakage is a factor which could also be the cause of a higher frequency of positive cultures at the second visit in earlier investigations. *Engström and Lundberg* (1966) showed that the change from a negative

culture to a positive could result between visits from leakage via the temporary filling. This risk was expressed in cases with considerable crown loss and especially if the period of time between treatments was more than 5 days. Their results were based on student material. In the authors material, the extent of crown loss was often considerable but great care was taken to make tightly sealed fillings and the chances of success were consequently greater than in Engström's material. In this material no dressings were put in the pulp cavity, but instead the fillings were inserted over a dry pellet. The time between visits varied from 2—20 days but there was no tendency towards a greater number of positive cultures after a longer interuption between the visits. A higher frequency of tightly sealed temporary fillings is the most likely explanation for the present findings differering from those of Engström and Lundberg.

The relatively high frequency of positive cultures after debridement (Di-samples), and at the second visit (S- or A-samples), (42 per cent resp. 34 per cent) is probably dependent on the relatively low number of molars in the material (31 per cent). *Engström and Frostell* (1962) showed that the relative frequency of bacteria free cases at the second visit is higher for molars than for one-rooted teeth, probably depending upon the longer time of effective treatment of each root for molars than for one-rooted teeth.

The average effective time for treatment in this material was 18 minutes. *Strindberg* (1965) reported a time of 27 minutes for treatment of one-rooted teeth. The longer treatment time in his material may explain the higher relative frequency of non-infected cases (76 per cent).

If only that part of the material, 64 cases, treated by one of the present investigators is studied, it is clear that the average treatment time was essentially longer (26 minutes) than in the entire material. Half of those cases consisted of molars. The relative frequency of sterile cases here was 77 per cent.

Results based on bacteriological examinations are often difficult to interpret, especially if they are old. The bacteriological sampling technique, substrates and the growth conditions are some variables which can lead to misinterpretations. Thus, this type of work will only allow for comparisons within the experimental series and no direct comparisons can be made with results obtained from other investigators.

In this work a sampling method described by *Möller* (1966) has been used. Brewer-medium with 10 per cent gelatin and 10 per cent serum has been used as a liquid medium. The addition of gelatin has *i.a.* a nonspecific inactivating effect on the antibacterial agents. Experiments have shown an equal frequency of bacterial growth in specimens taken from infected root

canals in this substrate when compared to thioglycollatemedium with 10 per cent inactivated horse serum. Thus, the risk for obtaining false negative cultures owing to the choice of medium is rather slight. The initial samples and the samples taken at the second visit have usually shown growth in the medium within the first three days. The samples taken after debridement rarely yielded growth before five days of incubation probably due to a reduced growth capacity of affected cells.

In judging the value of cultures from root canals the type of bacterial flora is a clue. *Möller* (1966) reported that the anaerobic group tends to increase and the aerobic group to decrease with improved conditions for sample taking and growing. The relative frequency of »other» microorganisms in the Di-samples was somewhat higher than in the other samples according to Table VII. This was caused by the cases where leakage occurred and where bacteria from the oral cavity had gained access to the operative area when the sample was being taken.

In the author's opinion the results clearly indicate that it is possible at the first visit after inactivation of the antibacterial agent, through a direct sample, to decide with satisfactory confidence whether the infection has been overcome or not. Thus the number of appointments can in all cases be reduced by one.

SUMMARY

The purpose of this study was to investigate if bacteriological examination of root canals made immediately after conservative treatment at the first appointment, could with satisfactory reliability, disclose whether infection had been eliminated or not. If this would be the case, such a sample could replace the one taken at the next appointment, thus reducing the overall number of visits by one.

After initial samples had been taken (I-samples) from the apical part of the root canals of 207 teeth, the canals were prepared mechanically with files and rinsed with hypochlorite solution. When preparation was finished the canals were dried and sodium thiosulfate solution was introduced for one minute as a specific inactivating agent against the antibacterial solution used. Bacteriological samples were then taken (Di-samples) after which sterile unprepared paper points were sealed into the teeth under temporary fillings.

At the second visit the enclosed paper points were removed for bacteriological examination (S-samples), and samples of the root canals were taken conventionally using a bacteriological sampling fluid (A-samples).

In 84.5 per cent of all cases the Di-samples showed agreement with samples taken at the second visit. The commonest causes for disagreement were in cases with positive Di-samples and negative A-samples (11.6 per cent of all cases). In 3.8 per cent of all cases negative Di-samples were followed by positive A-samples.

Thus, if one relies upon the Di-sample the risk of a persisting infection at a root canal filling procedure at the second appointment is 3.8 per cent.

RÉSUMÉ

EPREUVES BACTÉRIOLOGIQUES DES CANAUX RADICULAIRES DIRECTEMENT APRÈS TRAITEMENT CHIMIQUE ET MÉCANIQUE. ÉTUDE CLINIQUE ET BACTÉRIOLOGIQUE

Cette étude visa à déterminer si une épreuve bactériologique faite à la première séance immédiatement après la terminaison du traitement des canaux peut révéler avec une certitude suffisante si l'infection a été éliminée ou non. Si c'était le cas, un tel prélèvement pourrait remplacer un prélèvement fait pendant la séance suivante, ce qui permettrait de diminuer d'une séance la durée du traitement.

Nous avons fait un prélèvement bactériologique initial (prélèvement I) à la partie apicale des canaux radiculaires des 207 dents de cette étude. Les canaux ont ensuite subi le nettoyage mécanique et des irrigations avec une solution d'hypochlorite de sodium. Quand les canaux ont été considérés comme suffisamment préparés, nous les avons asséchés, après quoi une solution de thiosulfate de sodium à 5 % a été appliquée pour neutraliser la solution antiseptique. Un prélèvement bactériologique a ensuite été fait avec une solution spéciale (prélèvement Di). Des pointes de papier stériles et sèches ont été placées dans les canaux et la cavité fermée par une boulette de coton et un ciment provisoire.

Á la seconde séance, les pointes de papiers enfermées ont été enlevées pour être soumises à un examen bactériologique (prélèvement S). De plus un prélèvement bactériologique a été fait de la manière habituelle en utilisant une solution pour prélèvements (prélèvement A).

Dans 84,5 % de l'ensemble des cas, les épreuves Di ont été conformes aux épreuves de la seconde séance. Dans 11,6 % de l'ensemble des cas, des épreuves Di positives ont été suivies par des épreuves S ou A négatives. Dans 3,8 % de l'ensemble des cas, des épreuves Di négatives ont été suivies par des épreuves S ou A positives.

Ainsi, si on se base sur un résultat négatif du prélèvement Di, le risque qu'il persiste encore une infection lors de l'obturation des canaux à la seconde séance est de 3,8 %.

ZUSAMMENFASSUNG

BAKTERIOLOGISCHE PROBENAHME VON WURZELKANÄLE DIREKT NACH DER CHEMOMECHANISCHEN BEHANDLUNG

Die Untersuchungen bezweckten, die Möglichkeit zu untersuchen, ob die bakteriologische Probe, die während des ersten Besuches direkt nach der abgeschlossenen konservierenden Wurzelbehandlung genommen wurde, mit zufriedenstellender Sicherheit aufweisen kann, ob die Infektionen im Wurzelkanal eliminiert wurden oder nicht, das heisst, ob die Probenahme die nach der nächsten Behandlung vorgenommen wird, durch dieses Verfahren unnötig geworden ist, sodass die Behandlungsperioden um eine Sitzung verkürtz werden könnten.

Von sämtliche Wurzelkanälen der 207 Zähne, die diese Untersuchung umfasste, wurden initiale Bakterieproben (I-Proben) genommen, nachdem ein Zugang zu der Wurzelspitze geschaffen war. Die Wurzelkanäle wurden danach mechanisch unter Zufluss von Natriumhypochloritlösung gereinigt, wonach die Kanäle nach Trockenlegung mit 5 % tiger Natriumtiosulfatlösung aufgefüllt wurden, ein Präparat, welches als spezifisches Inaktivierungsmittel gegen die antibakterielle Spüllösung gilt. Proben mit specieller Lösung wurden danach genommen (Di-Proben). Die Kanäle wurden mit sterile unpreparierten Papierspitzen und temporären Füllungen zugeschlossen.

Bei der Zweiten Sitzung wurden die eingeschlossenen Papierspitzen für bakteriologische Untersuchung entfernt (S-Proben). Ausserdem wurden auf gewöhnliche Art bakterielle Proben genommen (A-Proben).

In 84,5 % sämtlicher Fälle zeigten die Di-Proben Übereinstimmung mit den Proben, die nach der zweiten Sitzung genommen wurden. Die meisten Fälle, die dazu beitrugen, dass eine Übereinstimmung nicht vorlag, waren Fälle mit positiven Di-Proben und negativen S- oder A-Proben. (11,6 % sämtlicher Fälle). In 3,8 % sämtlicher Fälle waren negativen Di-Proben von positiven S- oder A-Proben erfolgen.

Man kann daraus schliessen, dass man wenn man sich nach dem Resultat der Di-Proben richtet ein Risiko bei den Wurzelfüllen der zweiten Sitzung mit einer noch vorhandenen (nicht aufzuweisenden) Infektion von 3,8 % haben.

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