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EXPERIENCES OF BACTERIOLOGICAL ROOT CANAL CONTROL

by

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Bacteriological root canal control was proposed as early as 1901 by the American *Onderdonk*. In spite of the fact that a long time has elapsed since then, this objective method of deciding whether asepsis exists in the pulpal cavity is still not generally used in clinical routine. During recent years, however, an increasing number of dentists have had direct contact with the practical procedures for bacteriological root canal control. The initial contact with these methods occurred at dental schools and continuation courses. Thus, for example, in Sweden bacteriological sampling was introduced at the Royal School of Dentistry in Stockholm by *Strindberg* in 1951. At that time every student, in order to learn the technique of bacteriological controls, had to follow bacteriologically the treatment of three teeth without vital pulps. Commencing with the spring term of 1956 these controls were extended to cover all cases of treatment of pulpless teeth. Sampling, keeping of records, etc., were organized from the beginning in such a way that a later analysis of the material would be possible.

This report will present some of the results obtained from this work. The intention is to give the practitioner an idea of how bacteriological root canal control is carried out and how it affects the clinical work.

The question of a suitable medium for cultivation from root canals has most recently been dealt with at length by *Möller* (1960) in a study published after the present research was concluded.

A number of authors have shown that the frequency of samples with growth rises with the extension of the cultivation period. *Prader* (1949) maintained that after 2—3 weeks cultivation growth could still be obtained in isolated cases in samples from infected root canals. The majority of authors, however, consider that for practical clinical use a cultivation period of 2—3 days is sufficient (*Yates & Morse*, 1938; *Coolidge*, 1940, and others).

If the pulp cavity is open to the oral cavity it is always infected (*v. Amerongen et al.*, 1958, and others). Even with a closed pulp cavity and a non-vital pulp, however, infection can be shown in many cases. (*Brown & Rudolph*, 1957; *McDonald et al.*, 1957). Moreover, from necrotic pulps with gangrenous odour *Engström & Frostell* (1957) were able to show a higher frequency of growth than from necrotic pulps without odour in similar clinical material. Cultivations from previously root-filled teeth without areas of rarefaction which for various reasons were re-treated presented no growth in some instances. The necrotic pulpal tissue in teeth after pulpotomy appears in many cases to be infected (*Obwegeser et al.*, 1957). There is, however, a lack of major studies in which such material is reported. From pulpless teeth with areas of rarefaction, growth is shown in a higher frequency than in teeth without such change (*Sommer & Crowley*, 1940; *Morse & Yates*, 1941). *Van Amerongen and coworkers* (1958) published comprehensive investigations of the frequency of infection in teeth with different clinical diagnoses. Cultivations from 63 teeth with open, necrotic pulps revealed all to be infected while with 242 teeth with closed, necrotic pulps, 53 per cent were infected. Of 168 previously treated teeth, 36 per cent were infected. If roentgenological changes could be

shown in the periapical bone, the frequency of infection was higher than if such changes were absent.

Of great interest is the question as to whether different micro-organisms in infected root canals present varying degrees of resistance to therapeutic measures. In other words, it is important to establish which, if any, organisms constitute a problem for treatment. *Bender & Seltzer* (1952) reported that certain organisms constitute a problem in root canals treated with local antibiotics. These authors found that fungi and enterococci were more difficult to eliminate than other micro-organisms. In ionophoresis disinfection, *Guthof* (1953) indicated that enterococci were especially resistant.

MATERIAL

The material to be reported was collected at the Departments of Endodontics at the Royal Schools of Dentistry in Stockholm and Umeå during the years 1956—1959. Only patients treated by students are included in this study. All the teeth from which samples were taken lacked vital pulps. The teeth therefore were those with suppuration of pulps and necrotic pulps with or without gangrenous odour, as well as teeth with old root-fillings which for various reasons had to be re-treated. In all groups there are teeth with or without roentgenologically demonstrable periradicular changes.

METHODS

Root-canal technique

During the biomechanical instrumentation and irrigation of root canals a quaternary ammonium compound (Biosept® in a concentration of 1:1000) was used ad modum *Strindberg* (1956). The root canal was washed with alcohol, chloroform and Dakin's solution used separately. As a rule a solution of 5 per cent iodine in 10 per cent potassium iodine was sealed in the canal as a dressing between treatments.

Bacteriological technique

The bacteriological sampling was performed in a special sampling room equipped with ultra-violet lumination. Most of the controls were undertaken by the students themselves under the supervision of teachers. Exceptions were the so-called c-tests, which were always taken by teachers. When using liquid media for cultivation the technique proposed by *Strindberg* (1952) was employed. When utilizing solid media, the technique described by *Engström & Frostell* (1957) was applied.

Initial bacteria samples, i.e. samples from the pulp cavity at the first visit, were not taken. Thus the first bacteriological control was made at the second appointment, provided the root canal was prepared. These samples were called a-samples. If the a-sample showed bacterial growth a new sample, the b-sample, was taken on the next occasion. If growth occurred from this also, the following time a c-sample was taken, and so on. When growth no longer could be observed the tooth was, from a bacteriological point of view, considered ready for root-filling. Immediately before the root filling, a further bacteriological sample was taken. This was called the r-sample.

There are more b-samples than positive a-samples. The difference between b-samples and positive a-samples will therefore be an additional sum to the material. This as well as other discrepancies in similar materials are discussed in another paper (*Engström*, 1964).

The sampling material was transferred from the root canal to two fluid media. These were serum-dextrose broth (Ds) and Brewer's thioglycolate broth (Br) in different tubes. At the c-test and the subsequent samples, (excepting the r-test) solid media were also used. These were most often two blood-agar plates for aerobic and anaerobic cultivation but also blood agar with gentian violet was used for demonstrating enterococci.

In an experimental series comprising 348 teeth, four different media were used for each test: (1) Serum-dextrose broth, (2) Brewer's Thioglycolate Medium (Oxo Ltd., London), (3) Brain-Liver-Heart (Difco), and (4) Trypticase Dextrose Agar (Baltimore Biological Lab., Baltimore). The sampling was performed so that the operator could not see in what order the media were used.

The a- and b-samples were interpreted macroscopically. In case of doubt the sample was transferred to a new medium or smears were produced for microscopic examination. The test tubes containing the c-samples and subsequent samples, as well as the r-samples with growth, were sent to the Department of Oral Microbiology. The methods used for pure culture separation and identification of micro-organisms were those reported by *Engström & Frostell* (1957).

RESULTS

A. *Frequencies of samples with growth*

Tables 1 and 2 show the frequency of samples with growth in sampling to serum-dextrose broth and thioglycolate broth.

Table 1.

Results of a- and b-samples. Number of samples with growth.

	1 day		2 days		3 days		4 days		1 week	
	No.	%	No.	%	No.	%	No.	%	No.	%
a sample	130	55.3	184	78.3	213	90.6	226	96.2	235	100
b-sample	71	62 8	94	83.2	105	92.9	110	97.3	113	100

Table 2.

Results of c-, d-, and r-samples.

	Total no.	With growth		Growth demonstrated			
		No.	per cent	O	Ox	Ds	Br
c sample	97	49	50.5	39	42	44	46
d-sample	49	23	46.9	—	—	—	—
r-sample	899	128	14 2	—	—	—	—

O = aerobic blood agar

Ox = anaerobic blood agar

Ds = serum-dextrose broth

Br = Brewer's thioglycolate broth.

Of a total of 988 a-samples, 235 (23.8 %) showed growth in one or both tubes after one week's incubation. Corresponding figures for the b-samples were 263, of which 113 (43.0 %) were with growth. Of 97 c-samples, 49 (50.5 %) were positive. Of 899 r-samples, 128 (14.2 %) showed growth.

B. *Incubation period*

The material consisted of 235 a-samples and 113 b-samples which showed growth after one week's incubation (Tables 1 and 3; Fig. 1).

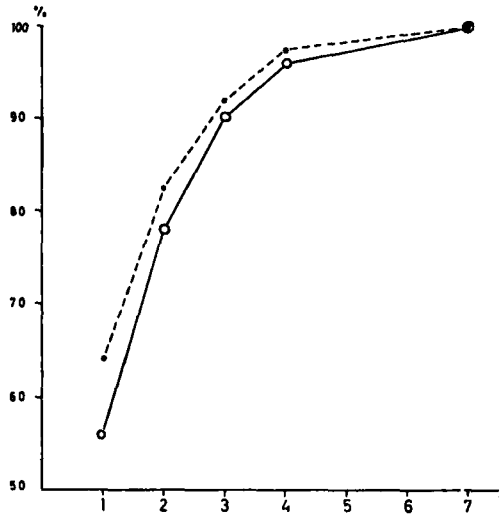

Fig. 1. Times for the appearance of turbidity in a- and b-samples with growth. After 4 days the growth frequency was 96.2 % (a-samples) and 97.3 % (b-samples), respectively. The incubation period in days is given on the x-axis.

o — o a-sample, • - - • b-sample.

Growth appeared in the serum-dextrose broth (Ds) after 2 days in 63.8 per cent and after 4 days in 75.3 per cent of the a-samples. Corresponding figures for b-samples were 75.2 and

Table 3.

Comparison between growth in serum-dextrose broth (Ds) and Brewer's thioglycolate medium (Br).

	1 day				2 days				3 days				4 days				1 week			
	Ds	Br	Growth in both	To-tal	Ds	Br	Growth in both	To-tal	Ds	Br	Growth in both	To-tal	Ds	Br	Growth in both	To-tal	Ds	Br	Growth in both	To-tal
a-sample	103	112	85	130	150	163	129	184	168	181	136	213	177	191	142	226	182	198	145	235
b-sample	65	58	52	71	85	79	70	94	90	88	73	105	95	93	78	110	96	97	80	113

Ds = serum-dextrose broth

Br = Brewer's thioglycolate medium

84.1 per cent, respectively. When two different media were used — Ds and Br — the corresponding figures rose to 78.3 and 96.2 per cent and 83.2 and 97.3 per cent, respectively.

Of 323 samples cultivated in the two above-mentioned media which after one week were interpreted as negative (from one series with a total of 516 samples), 8 (2.5 %) proved to be positive (*i.e.* 1.6 % of the original tests), during the continued incubation up to three weeks. In all these cases growth was obtained in only one of the tubes.

C. *Comparison between different media*

Table 3 and Fig. 2 show a comparison of growth frequencies between serum-dextrose broth and Brewer's thioglycolate medium when samples from each tooth were transferred to these

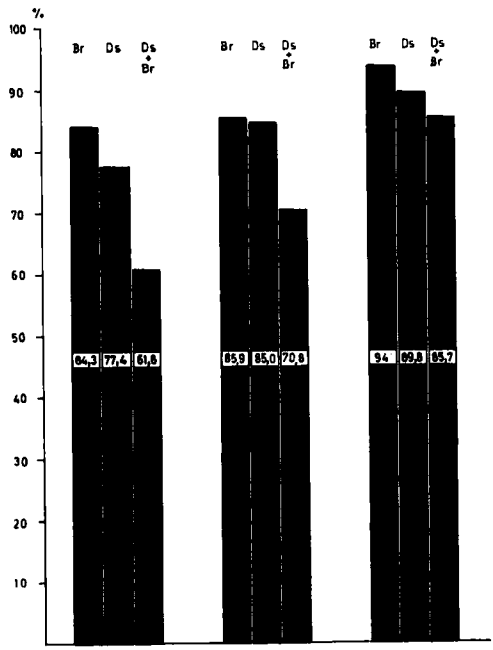

Fig. 2. Frequency of growth in serum-dextrose broth (Ds) and thioglycolate medium (Br) and the frequency of simultaneous growth in Ds and Br in samples where growth was shown. The groups of columns show, starting from left, the a-, b-, and c-samples.

Table 4 a.

Growth frequency in four different media used in combination. Total number of samples 348, of which 144 gave growth in one or more media.

Medium	Growth	%
Ds	97	67.4
Br	115	79.9
HHL ...	102	70.8
Tr	92	63.9

Ds = serum-dextrose broth
Br = Brewer's thioglycolate medium
HHL = brain-liver-heart medium
Tr = trypticase dextrose agar.

Table 4 b.

Growth frequency in the different media and combinations of media.

Ds Br H H L Tr	Ds	Br	H H L	Tr	Ds Br	Ds H H L	Ds Tr	Br H H L	Br Tr	H H L Tr	Ds Br H H L	Ds Br	Ds H H L Tr	Br H H L Tr	Total
72	11	16	8	4	3	3	2	6	5	1	5	1	—	7	144

Table 4 c.

Frequency of samples with growth which would have been demonstrated with different combinations of 2 media.

Ds and Br	131	91.0 %
Br and HHL ...	127	88.2 %
Br and Tr	122	84.7 %
Ds and HHL ...	119	82.6 %
and Ds Tr	114	79.2 %
HHL and Tr ...	114	79.2 %

Ds = serum-dextrose broth
Br = Brewer's thioglycolate medium
HHL = brain-liver-heart medium
Tr = trypticase dextrose agar.

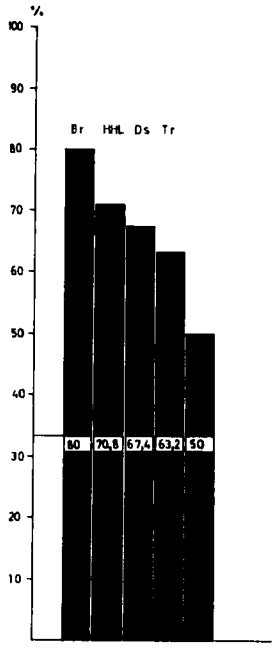

Fig. 3. Frequency of growth in four different standard media upon simultaneous sampling to all four. The column on the extreme right indicates the growth in all four media.

two media. The total number of a-samples was 988. After one week growth was shown in 182 Ds-tubes and 198 Br-tubes. Growth in both media occurred in 145 cases. The corresponding figures for 263 b-samples were 96, 97 and 80.

Tables 4 a, b and c as well as Figs. 3 and 4 show the results of an experimental series in which samples were taken to four different liquid media simultaneously. In addition to the two mentioned earlier (Ds and Br), brain-liver-heart medium as well as trypticase dextrose agar were used. The greatest growth frequency (80 per cent) was obtained with thioglycolate medium, and of varying combinations of two media, the combination Ds and Br showed the greatest growth frequency (91 per cent positive samples).

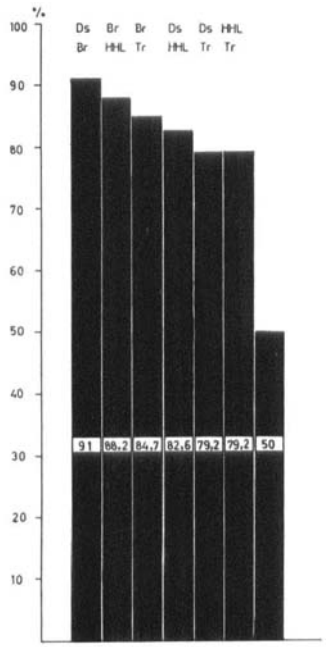

Fig. 4. Frequency of growth as determined in combinations of standard media. Samples taken to four media simultaneously (Fig. 3). The combination Ds and Br gave the greatest growth frequency. The column on the extreme right gives the simultaneous growth in all four media.

D. *Frequency of samples with growth in relation to different pulp and periapical bone diagnoses* . .

The material in this part of the study consists of 1170 a-samples of which 322 (27.5 %) showed growth. Corresponding values for b-samples were 352 and 143 (40.6 %), respectively. Table 5 shows the extent of persisting infection in teeth with different pulpal and periapical bone diagnoses in a-samples. Fig. 5 also shows the frequency of persisting infection as indicated by positive a-samples related to different groups of teeth.

Persisting infection occurred in a higher frequency in teeth with non-vital pulp (necrosis or gangrene) than in teeth with previous root-fillings ($P < 0.001$). Persisting infection also occurred rather more frequently in incisors with roentgenologically observable pathological changes in the periradicular bone than in incisors without such changes. The differences, however, were not statistically significant. There was less tendency for persisting infection in multi-rooted teeth than in single-rooted teeth.

Table 5.

Frequency of samples with growth from different tooth groups and different pulp diagnoses.

	N+			N−			R+			R−			Total		
a-samples	No.	Of which with growth	%	No.	Of which with growth	%	No.	Of which with growth	%	No.	Of which with growth	%	No.	Of which with growth	%
I	92	43	46.7	77	23	29.9	114	29	25.4	170	25	22.7	393	120	30.5
P	84	31	36.9	109	36	33.0	96	20	20.8	194	41	21.1	483	128	26.5
M	85	23	27.1	111	32	28.8	25	5	20.0	73	14	19 2	294	74	25.2
Total	261	97	37.2	297	91	30 6	235	54	23.0	377	80	21.2	1170	322	27.5

N = necrosis-gangrene
R = previously root-filled
\+ = periradicular rarefaction
— = no periradicular rarefaction
I = incisors
P = premolars
M = molars

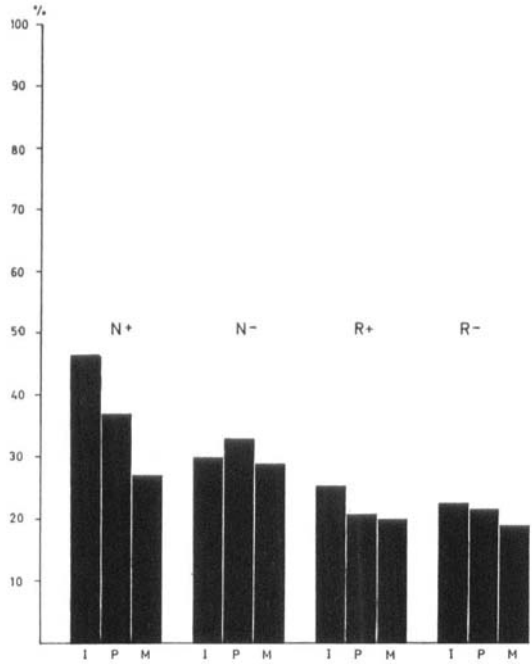

Fig. 5. Frequency of samples with growth in relation to pulp and periradicular bone diagnoses and tooth group.

N = necrosis-gangrene
R = previously root-filled
+ = periradicular rarefaction
— = no periradicular rarefaction
I = incisors
P = premolars
M = molars

E. *Micro-organisms isolated in c-samples and r-samples*

From 49 teeth with positive c-samples, 72 different strains of micro-organisms were isolated. These were found to be pure cultures in 36 (73.5 %) of the 49 teeth.

From 103 teeth with positive r-samples, 126 different strains of micro-organisms were isolated. In 84 (81.6 %) of these 103 teeth the organisms were found in pure culture (Table 7).

From Tables 6 and 7 it can be seen that aerobic micro-organisms predominate as compared with anaerobic ones. Streptococci occur most commonly. In the c-samples, enterococci pre-

Table 6.

Frequency of different micro-organisms in c-samples.

Micro-organism		Pure culture
Staphylococcus epidermidis (albus)	2	2
Staphylococcus aureus	1	—
Gaffkya tetragena	2	—
Sarcina lutea	2	1
Alpha-streptococci (viridans group)	15	6
Gamma-streptococci (viridans- and lactis groups)	14	7
Enterococci (not differentiated)	4	3
Streptococcus faecalis	11	8
„ „ var. liquefaciens	2	1
„ „ var. zymogenes	1	1
Enterococci total	18	13
Anaerobic streptococci	3	2
Neisseria	2	—
Veillonella	2	—
Diphtheroids	3	—
Grampositive rods (subtilis?)	1	1
Escherichia coli	1	1
Coliform bacteria	2	—
Pseudomonas aeruginosa	2	2
Yeasts	2	1
Total:	72	36 (73.5 %)
No. of teeth:	49	

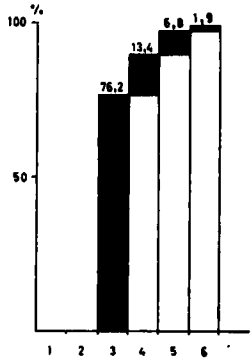

Fig. 6. Frequency of teeth which could be root-filled at the third, fourth, fifth, and sixth appointments respectively. After six appointments there remained 1.7 % with persisting growth.

dominate among the latter but in the r-samples, alpha-streptococci are most numerous. Apart from Staphylococcus epidermidis noted in the r-samples, other micro-organisms listed in the tables occur sporadically. It is, however, striking that anaerobic streptococci are encountered rather frequently in these samples.

Table 7.

Frequency of different micro-organisms in r-samples.

Micro-organism		Pure culture
Staphylococcus epidermidis (albus)	10	7
Staphylococcus aureus	3	—
Micrococci, Gaffkya tetragena	4	2
Sarcina lutea	1	1
Alpha-streptococci (viridans group)	35	23
Gamma-streptococci (viridans- and lactis groups)	21	11
Enterococci (not differentiated)	3	3
Streptococcus faecalis	12	10
„ „ var. liquefaciens	1	1
„ „ var. zymogenes	1	—
Enterocci, total	17	14
Anaerobic streptococci	12	9
Lactobacilli	2	1
Diphtheroids	3	3
Aerobic grampositive sporeforming rods	5	5
Atypical grampositive rods	2	2
Pseudomonas aeruginosa	2	1
Yeasts	9	5
Total:	126	84 (81.6 %)
No. of teeth:	103	

DISCUSSION

The present study is an analysis of routine root canal controls carried out by students at a teaching institution. In many instances the sampling took place under the supervision of teachers, but in others there was no supervision. It can be presumed that the students do not have the same clinical ability as prac-

tising dentists. It is probable that the frequency of contamination is higher with students than if experienced practitioners had secured the samples. It must also be presumed for other reasons that the frequency of samples with growth is higher than it would be if the investigation had been undertaken by a clinician with a special interest in the subject. It may, however, be assumed that the careful control and supervision which characterizes the procedures in departments of endodontics to some extent compensates for the circumstances mentioned. An advantage of student material is, moreover, that within a reasonable time it becomes of sufficient extent to be feasible for analysis.

As a result of inter alia the investigations of *Möller* (1960) the question of a suitable medium for cultivation of samples from root canals has been further illuminated. An investigation carried out with due regard to this information may therefore be expected to show growth in a higher frequency than that reported in the present work.

In a majority of instances, an experienced clinician manages to conclude the biomechanical preparation of the root canals at the first visit and therefore is ready to take the a-sample during the second appointment. It should be pointed out that owing to insufficent experience on the part of the students, a tooth in this investigation may have been treated several times before the first bacteriological sample was obtained; thus more than one dressing may have been applied before the taking of this sample. The material presented in this report is, from this point of view, heterogeneous. Certain conclusions of general clinical interest can, however, be drawn from the investigation.

The comparison of efficiency of different media indicates that the combination of serum-dextrose broth and thioglycolate medium was suitable, having regard to the media which were commercially easily available at the time in question.

The length of the incubation period is, among other factors, to be also considered with what media are used. The two media used in this investigation, (used also by the majority of clinicians who themselves carry out bacteriological root canal control) require an incubation period of at least 4 days.

Owing to the fact that there appears to be at the present time a certain amount of confusion in regard to media, it should be pointed out that (1) an attempt must be made to carry out the sampling with an anaerobic medium to which serum has been added (Brain-Liver-Heart medium or thioglycolate medium with horse serum added), (2) a combination of two anaerobic media with somewhat different qualities — one with and one without serum — seems to give a higher frequency of samples with growth than either of the media alone, and (3) even at teaching institutions it is not possible to use a combination of media which gives maximum frequency of samples with growth. The fact that it may be difficult to procure the most ideal cultivation facilities is no reason for rejecting bacteriological root canal control as such. Bacteriological root canal control carried out with the generally available standard media used in the present study has proved to be of great clinical value.

Of great interest is the difference in the frequencies of samples with growth in a- b- and c-samples (27.5 %, 40.6 % and 50.5 %) respectively. The demonstration of growth in the a-sample shows that some factors have impeded the antibacterial treatment of the pulp cavity, which, in the majority of cases, succeeds with a single treatment. It should also be pointed out that in a certain percentage of the cases no infection primarily occurred in the pulp cavity especially in many re-treated cases. The high frequency figures in the b- and c-samples show that in nearly half of the cases the factor mentioned was not overcome or revealed at the taking of the sample. Continued growth may be caused by contamination from outside (e.g. from the air or by using non-sterile instruments) or by faulty technique in securing the samples. The frequency of samples with growth arising from this cause can, however, be kept low. Undoubtedly a more common factor is contamination of the pulp chamber by fluids from the oral cavity. This may be caused by an inefficient seal of the temporary filling or difficulties in applying the rubber dam, e.g. in the case of deep proximal cavities. If caries and old fillings are not removed completely or if a root canal in multi-rooted teeth is not found and cleaned, persisting infection is often shown. It was proved that, in the series with growth in a- and b-samples and so on, such cases are rather common. Sometimes

root cracks which are difficult to detect have been found to be the reason why asepsis could not be achieved. In several cases, communication with the sinus maxillaris was revealed through persisting growth in the bacteriological controls. If growth is shown at the taking of the first sample (a-sample) this should be regarded as a warning to intensify the control of technique in order to be able to discover possible reasons for the failure of the treatment. By virtue of experience we know now that if the bacteriological analysis in the c-sample has shown growth of mixed flora of oral bacteria, (e.g. alpha-streptococci, Neisseria, lactobacilli, yeasts etc.) it is almost certain that leakage occurs. Two cases may illustrate this:

A maxillary first premolar with two root canals with previously mummified pulp tissue was fitted with a copper ring prior to treatment due to large carious lesions. This ring was attached with zincphosphate cement. After two samples yielding growth, a c-sample was taken. From the solid medium the following organisms could be isolated in spite of high colony frequency: alpha-streptococci, Sarcina, Gaffkya, difteroids and yeasts. The patient was recalled and a new copper ring was cemented on. The canals were washed and irrigated. As previously, a 5 per cent solution of iodine in 10 per cent potassium iodine was used as dressing. A bacteriological sample was taken after five days. This sample and one taken on the occasion of the root-filling showed no growth.

Upon treatment an incompletely root-filled maxillary second premolar with periapical osteitis and deep proximal caries gave growth in the a- and the b-samples. The c-sample gave pure culture of yeasts. The d-sample gave gamma-streptococci as well as gram-positive rods and threads. In the next sample, alpha- and gamma-streptococci, Neisseriae, Staphylococcus epidermidis and yeasts were obtained. On the following visit, pure cultures of yeasts were again obtained. On this occasion a copper ring was cemented on, the root canal was treated again and the same antiseptic as utilized earlier was sealed in the canal. The sample taken at the next appointment, like the r-sample, gave no growth.

In none of these cases could contamination from the saliva be demonstrated clinically.

It is most convenient to work without copper rings. If they

are used it is necessary to be extremely careful when cementing them on. The rings must be cut and contoured as for taking impressions. Before cementation, it is necessary to have a clear idea of the anatomy of the pulp cavity; for in numerous instances, after removing copper rings, it has been found that the ring or the cement filling has masked one of the root canal openings.

If the frequency of bacterial growth in the a-samples is compared to previously ascertained pulpal diagnoses, we find the frequency to be lower (highly significant) in the case of teeth with old root canal fillings which are being re-treated than in teeth with pulpal necroses (with or without gangrenous odour). An explanation may be that previously root-filled teeth were initially bacteria-free in a higher percentage than other teeth. The re-treatment was carried out due to the fact that the previous root filling appeared inadequate in the roentgenogram. When re-treating teeth with old root fillings it may save time to take initial cultures, for these often show that there is no infection in the pulp cavity. The tooth can then be rootfilled at the second visit.

With roentgenologically demonstrable osteitis, the frequency of a-samples with growth is greater than if no roentgenological changes exist. This is especially true of incisors. The difference, however, is not statistically valid. Even if this tendency were confirmed through study of a larger material, it cannot, however, be taken as evidence that an infection is easier to eliminate from a multi-rooted than from a single-rooted tooth. It is possible that the taking of a satisfactory sample is more difficult in multi-rooted teeth (i.e. narrow canals, paper-point not penetrating to the apex, samples from apical ramifications absent). This tendency is interesting, however, when related to the better healing results shown by *Strindberg* (1956) in the treatment of multi-rooted teeth than of single-rooted teeth.

With local antibiotic treatment of infected root canals, certain micro-organisms have proved to be particularly resistant, e.g. enterococci and fungi (*Bender & Seltzer,* 1952). Also, with electrolytic medication enterococci have proved to be resistant (*Guthof,* 1953). In the present material, which had been subjected to conservative treatment with conventional root canal

medicaments, a high frequency of *enterococci* was shown among those cases which were not bacteria-free after treatment.

Strains of *Pseudomonas aeruginosa* and of *Escherichia coli* also proved difficult to eliminate. They could be shown in a number of samples after the c-sample and then, usually in pure culture. From Fig. 6 it can be seen that 1.7 per cent of the teeth could not be root-filled due to persistence of growth even after six treatments. As a rule the growth was, in these cases, a pure culture of one of the above-mentioned micro-organisms. It is striking that anaerobic streptococci recur in quite a high frequency in the r-samples.

Thus in certain cases the difficulty of obtaining asepsis in infected root canals is caused by the presence of particularly resistant bacteria such as enterococci and gram-negative, rod-shaped enteric bacteria. It is important that such cases be revealed as soon as possible and undergo special treatment. This can either be done through type determination of initial samples or, to reduce the burden on the laboratory, by immediate type determination of samples with growth. The present investigation thus points to the value for the clinician of collaboration with a bacteriological laboratory.

In the American literature it is often recommended that two consecutive negative cultivations should take place before a root filling. In the present investigation, micro-organisms could be cultured in 14.2 per cent of the r-samples. Some of these positive samples were undoubtedly caused by deficiencies in the aseptic procedure. This hypothesis is supported by the occurrence of *Staphylococcus epidermidis*, which is not normally found with the typical root canal flora. Other positive tests may have been caused by a defective seal of temporary cement. In the majority of cases, however, the preceding negative samples do not seem to have reflected the actual conditions in the pulp cavity. The frequency is strikingly high and positively justifies a new attitude of ours to the question of the number of necessary controls. In a following research report this problem will be considered.

The greatest value of bacteriological root canal control is that (apart from inevitable erroneous results) it indicates whether asepsis in the pulpal cavity has or has not been achieved. Experience shows that no other clinically usable method can at

present replace bacteriological control and that it often aids in avoidance of serious treatment errors. It encourages increased watchfulness and care and by so doing has greatly contributed to a refinement of endodontic techniques.

It is, however, still an open question whether the results of such refined endodontic techniques justify the supplementary measures and costs entailed by bacteriological root canal control. The clinicians with a special interest in endodontics, who have been accustomed to using bacteriological root canal control, will undoubtedly answer in the affirmative, due to the large number of errors that can thus be avoided. Other clinicians may wish to have the value of the method documented in analytical studies based on radiographic and clinical follow-up examinations before they are prepared to undertake the extra effort involved. The authors hope to deal with these problems in subsequent investigations.

SUMMARY

In the present paper the experience of bacteriological root canal control at the Royal Schools of Dentistry in Stockholm and Umeå are reported. During conservative treatment of pulpless teeth performed by the students, samples were taken to different liquid media in order to determine whether asepsis of the canals was or was not obtained by the treatment. In instances when growth was present in spite of prolonged treatment, samples were taken to solid media in order to determine the organisms present in the pulpal cavity.

The first sample (a-sample) was obtained when the mechanical preparation of the canal was finished (i.e. in most instances at the second visit). In about 75 per cent of the cases no growth was obtained and the roots were filled at the next (third) appointment. In instances where growth was present, a new sample (b-sample) was obtained. Of the b-samples, growth was obtained in about 40 per cent. If growth was present in the b-sample, a new sample (c-sample) was obtained. The c-samples were transferred to both liquid and solid media. The frequency of growth in the c-samples was about 50 per cent.

It was found that if samples were taken to several liquid media, the frequency of growth was higher the more media were used. An evaluation of different combinations of four commercially obtainable products showed that in the case of combinations of two media, the highest frequency of growth was obtained when samples were taken to both serum-dextrose broth and thioglycolate broth. It is generally believed that serum-dextrose broth favours the growth of aerobic micro-organisms and that the thioglycolate broth enhances that of anaerobes.

It was shown that the period of incubation must be at least four days if this combination of substrates is used. After four days, growth had appeared in 97 per cent of those samples that were considered positive after one week.

Among the cases in which growth was persistently obtained, there was a high percentage of teeth with deep proximal cavities, root canals which had not been discovered, root cracks etc. (i.e. cases in which leakage from the saliva or from some other area of infection was probable). Thus, if growth was obtained in the a-sample, a thorough inspection of the tooth was made to find leakage, caries, or other circumstances the presence of which would often not have been suspected if a bacteriological test had not been performed.

In some of the cases with persistent growth, pure cultures of resistant organisms were found, especially cultures of enterococci but in some instances of *E. coli* or *Pseudomonas aeruginosa*. In these cases, the treatment was directed against the particular organism noted for example by determining its sensitivity to different antibiotics.

An analysis of the frequency of growth from different types of teeth and from teeth with different diagnoses revealed that growth was obtained more often from teeth with necrosis or gangrene of the pulp than from teeth which were previously root-filled (difference highly significant). Furthermore, there was a tendency for persistent infections to be more common in single-rooted teeth than in multi-rooted teeth and for growth to be present more often from teeth with a periapical area of rarefaction than from teeth without such changes. These differences, however, were not statistically significant.

RÉSUMÉ

EXPÉRIENCE ACQUISE EN MATIÈRE DE CONTROLE BACTÉRIOLOGIQUE DES TRAITEMENTS RADICULAIRES

Le présent article rend compte des expériences faites dans les Ecoles Dentaires Royales de Stockholm et d'Umeå sur le contrôle bactériologique des canaux radiculaires. Au cours du traitement conservateur de dents dépulpées effectué par les étudiants, des prélèvements ont été mis en culture dans divers milieux liquides dans le but de déterminer si l'asepsie des canaux avait été obtenue par le traitement ou non. Dans les cas où, malgré un traitement prolongé, la culture était positive, des prélèvements ont été mis en culture dans des milieux solides afin d'identifier les germes présents dans la cavité pulpaire.

Le premier prélèvement (prélèvement a) était fait à la fin de la préparation mécanique du canal (c.à.d. en général à la deuxième séance). Dans 75 p. 100 des cas, la culture était négative, et l'obturation radiculaire était effectuée à la séance suivante (troisième séance). Dans les cas où la culture était positive, on faisait un nouveau prélèvement (prélèvement b). La culture de ces prélèvements b était positive dans 40 p. 100 des cas. Lorsque les prélèvements b donnaient une culture positive, on faisait un nouveau prélèvement (prélèvement c). Les prélèvements c étaient mis en culture dans des milieux liquides et dans des milieux solides. La fréquence des cultures positives à partir des prélèvements c était d'environ 50 p. 100.

Il est apparu que lorsque des prélèvements étaient mis en culture en même temps dans différents milieux liquides, la fréquence des cultures positives était d'autant plus élevée qu'on utilisait un plus grand nombre de milieux. Une comparaison de différentes combinaisons de quatre produits se trouvant dans le commerce a montré que dans les cas de combinaison de deux milieux, la fréquence de cultures positives la plus élevée était obtenue lorsque les prélèvements étaient mis en culture à la fois dans du bouillon sérum glucosé et dans du bouillon au thyoglycolate. Le bouillon sérum glucosé est généralement considéré comme favorisant la culture des germes aérobies, et le bouillon au thyglycolate comme favorisant la culture des germes anaérobies.

La période d'incubation nécessaire lorsque cette combinaison de milieux est utilisée s'est révélée être d'au moins quatre jours. Au bout de quatre jours, les cultures étaient déjà positives pour 97 p. 100 des prélèvements qui furent considérés comme positifs au bout d'une semaine.

Parmi les cas dans lesquels une culture positive continuait à être obtenue, il y avait une proportion importante de dents présentant des cavités proximales profondes, des canaux radiculaires non décelés, des fêlures radiculaires, etc. (c'est-à-dire des cas où il existait probablement un défaut d'étanchéité laissant pénétrer une infection provenant de la salive ou d'autre source). Aussi, lorsque le prélèvement a donnait une culture positive, il a été procédé à un examen attentif de la dent pour déceler tout défaut d'étanchéité, carie, ou autre détail dont la présence n'aurait le plus souvent pu être soupçonnée en l'absence d'un examen bactériologique.

Dans certains des cas présentant une persistance de la positivité des cultures, des cultures pures de germes résistants ont été trouvés, en particulier des cultures d'entérocoques, mais dans quelques cas des cultures d'*Escherichia coli* ou de *Pseudomonas aeruginosa*. Dans ces cas, le traitement a été dirigé contre le germe en question caractérisé pa exemple par une détermination de sa sensibilité à différents antibiotiques.

Une analyse de la fréquence des cultures positives obtenues à partir de différents types de dents ou à partir de dents représentant différents diagnostics a montré que les cultures étaient plus souvent positives dans les cas de dents présentant une nécrose ou une gangrène pulpaire que dans les cas de dents soumises antérieurement à une obturation radiculaire (différence hautement significative). De plus, il existait un tendance indiquant que les infections persistantes étaient plus fréquentes dans les dents monoradiculaires que dans les pluriradiculaires, et que les cultures étaient plus souvent positives dans les cas de dents présentant une zone de raréfaction péri-apicale que dans les cas où il n'y avait pas de raréfaction. Ces différences n'étaient cependant pas significatives du point statistique.

ZUSAMMENFASSUNG

ERFAHRUNGEN MIT BAKTERIOLOGISCHER WURZELBEHANDLUNGS-KONTROLLE

In der vorliegenden Arbeit werden Erfahrungen mit bakteriologischer Wurzelbehandlungskontrolle an den zahnärztlichen Hochschulen in Stockholm und Umeå dargelegt.

Bei konservierenden Wurzelbehandlungen wurden Proben nach einem besonderen Schema mit verschiedenen fliessenden Substraten genommen, mit der Absicht festzustellen, ob die Infektion im Wurzelkanal überwunden war oder nicht. In gewissen Fällen, wo trotz wiederholter Behandlung weiterhin Wachstum bestand, wurden Proben auf festes Substrat übertragen, wobei eine Typbestimmung von eventuell wachsenden Organismen vorgenommen wurde.

Eine erste Probe (a-Probe) wurde bei der ersten Behandlung genommen, nach der Sitzung, bei der die Präparation des Kanales vollendet wurde, im allgemeinen also bei der zweiten Behandlung.

In ungefähr 75 % der Fälle war dann die eventuelle Infektion überwunden, nach den Beurteilungsmethoden, die angewendet wurden, und die Wurzelfüllung konnte meistens nach der dritten Behandlung durchgeführt werden.

In Fällen mit Wachstum wurde eine neue Probe genommen (b-Probe). Bei der b-Probe wurde Wachstum in ungefär 40 % der Fälle konstatiert. Bei Wachstum in der b-Probe wurde eine weitere Probe genommen (c-Probe), nun auf sowohl festem als auch fliessendem Substrat. Die Wachstumsfrequenz bei der c-Probe war ungefär 50 %.

Es zeigte sich, je mehr fliessende Substrate angewendet wurden, desto grösser war die Frequenz der Fälle, bei denen Wachstum nachgewiesen werden konnte.

Bei der Untersuchung verschiedener Kombinationen von vier im Handel zugänglichen Substraten wurde die höchste Anschlagsfrequenz erhalten, wenn Serumdextrosebouillon und Tioglykolbouillon angewendet wurden. Es wird angenommen, dass Serumdextrosebouillon aerobe Organismen begünstigt, während Tioglykolbouillon anaerobe Organismen fördert. Es zeigte sich

weiter, dass die Inkubationszeit mindestens drei mal 24 Std. sein sollte, wenn man die oben genannten Substrate anwendet. Nach dieser Zeitspanne war die Häufigkeit der Proben mit Wachstum 97 % der Frequenz der Proben, die nach einer Woche konstatiert worden waren.

Unter den Fällen, bei denen Wachstum nachgewiesen wurde, und besonders, wenn Wachstum wiederholt nachweisbar war, befand sich eine Anhäufung von Zähnen mit tiefen, apprixomalen Kavitäten, nicht entdeckten Wurzelkanälen, gesprungenen Wurzeln u.s.w., welche eine Leckage verursachen können.

Wachstum in der a-Probe gab also Veranlassung den Zahn genau zu untersuchen, um eventuelle Leckage oder Restkaries oder andere Defekte zu entdecken, die oft nicht unter anderen Umständen ans Licht gekommen wären.

In gewissen Fällen mit bestehendem Wachstum wurden Reinkulturen von besonders beständigen Organismen nachgewiesen, vor allem Enterokokken, aber in einem Fall ebenfalls E. coli und Pseudomonas aeruginosa. In diesen Fällen konnte die Behandlung speziell auf die vorkommenden Organismen eingerichtet werden, z.B. nach der Bestimmung der Empfindlichkeit gegen Antibiotika.

Bei der Frequenzanalyse von Proben mit Wachstum bei verschiedenen Zahngruppen und verschiedenen Pulpa- und Kieferknochendiagnosen zeigte es sich, dass Wachstum seltener von früher wurzelgefüllten Zähnen erhalten wurde, als von Zähnen mit nekrotischer Pulpa (stark signifikant). Ausserdem war eine Tendenz vorhanden, dass eine bestehende Infektion häufiger an einwurzligen Zähnen war, als an mehrwurzligen, sowie, dass Wachstum öfter in Proben von Zähnen mit röntgenologisch nachweisbaren periapikalen Veränderungen vorkam, als an Zähnen, die keine solche Veränderungen hatten.

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