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BACTERIOLOGIC RESULTS FROM 4,000 ROOT CANAL CULTURES

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INTRODUCTION

THE root canal culture is, in the first place, a test for sterility. Consequently, it is of use only if careful aseptic and endodontic techniques are practiced. Aseptic technique is, nevertheless, requisite to endodontic treatment. If only as a check on aseptic technique, root canal culture is of important educational value.

The results of culturing depend partly on the medium used for primary culture.^{7, 11, 14, 16} In a previous publication,¹ we compared various media. Brain-heart infusion with 0.05 per cent agar in screw-capped bottles proved quite satisfactory and permitted the growth of most common aerobic and anaerobic organisms.

Whether or not the root canal culture gives a true picture of the bacteriologic situation in the canal has been much discussed. It has been claimed, on the basis of theoretical considerations, that a negative culture from a positive canal would be a regular occurrence. Many authors, therefore, require two consecutive negative cultures before they will accept sterility of the canal.^{5, 8, 15} Van Amerongen² showed, however, that with a suitable technique the probability for a positive culture after one or after two negative cultures is equal and low (2 to 3 per cent for experienced operators; 8 to 9 per cent for students). The probability that the canal is sterile is not higher after two negative cultures than after one negative culture. Furthermore, large masses of dentine filed from the wall of a well-prepared canal generally were sterile if the normal paper-point culture had been sterile, giving further proof that negative cultures from root canals are representative in practice. The number of false positives is fairly high, of course, but it decreases with experience in aseptic techniques.^{2, 9}

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A high percentage of infected canals become sterile by mechanical preparation of the root canal.^{6, 17} In our experience, this percentage was 64 per cent.³ This phenomenon stresses the view that what you take out of the canal is at least as important as what you put into it. In this connection, a comparison was made of the results obtained with chlorophenol-camphor-menthol and polyantibiotics.⁴ The results showed that although the number of applications necessary to obtain sterility was slightly smaller in the group treated with polyantibiotics, the mean number of sessions was equal in both groups.

Regular culturing from root canals, starting with an initial culture during the first session, reduces the number of sessions necessary for treatment. The culture provides an invaluable check on the careful aseptic technique which is required for good root canal treatment. Without this regular check (that is, with less careful technique), the number of infected canals becoming sterile by preparation alone would be much less than 64 per cent. This leads to the remarkable paradox² that root canal cultures are important for those who do not practice them and much less so for those who do. That a sound knowledge of the bacterial flora in infected canals is of importance in cases where focal infection is considered needs no comment.

Having advocated the practice of root canal cultures, we wish to report on the bacteriologic results of more than 4,000 cultures from more than 1,000 canals.

MATERIALS AND METHODS

Endodontic Techniques.—These have been described before.¹ After extensive mechanical cleansing of the exterior of the tooth, the rubber-dam was applied (when necessary, after insertion of a temporary inlay). The tooth in question was disinfected by the application of 5 per cent tincture of iodine. After the removal of all carious dentine this disinfection was repeated. The roof of the pulp chamber and the coronal pulp were then removed with sterile instruments; they were flushed repeatedly with sterile saline. For initial cultures, a sterile paper point was brought in contact with the contents of the canal. For initial cultures from cases of old endodontic treatments, periapical secretion was collected and dentine particles were filed from the canal wall. After mechanical preparation, some dentinal material was filed from the wall of the dry canal and a paper point was introduced, care being taken to make contact with the periapex. The paper point was left in situ for three minutes, wet with sterile saline, and transferred to the culture bottle. After the application of drugs, the canal was washed with 2 ml. of sterile saline. It has been shown¹ that this technique prevents growth inhibition of the used medicaments.

Most cultures were taken by advanced dental students; a number of cases were treated by experienced dentists on the staff of our dental school.

Bacteriologic Procedures.—Primary cultures were taken in screw-capped 5 ml. bottles filled with brain-heart infusion broth (Difco) to which 0.05 per

cent of agar had been added (B.H.I.) All cultures were incubated at 37° C. As soon as turbidity was observed, a microscopic preparation was made and the fluid was subcultured on three (later four) solid media. One blood agar plate was incubated aerobically. One blood agar plate and a Rogosa medium (pH 6.8) were incubated anaerobically. A Chapman plate, incubated aerobically, was added at a later period. In many cases these plates showed pure cultures. From the colony form and stained slides, a rough classification could be made. This was then confirmed, depending on the case, by short sets of biochemical tests or by serologic tests. From mixed infections, different colonies were picked and subcultured for further identification.

Identification was, of necessity, preliminary. Important strains were kept for further study. With growing experience, it became possible (even with simple means) to allot each isolated strain to a definite group.

It is recognized that in the fluid primary culture some organisms might be selected and others suppressed. To arrive at more quantitative results, we plated the paper points directly and in another series triturated the paper points in broth with sterile sand and plated the resulting fluids. As neither of these experiments was encouraging, we adhered to the technique described.

The properties used for identification of the isolated strains will be described with each organism. Serologic grouping of streptococci was accomplished by Fuller extraction and precipitation in capillary tubes with commercial sera.

During a certain period of the study the value of two different culture media for primary culture was studied by taking duplicate cultures from each canal. It has been shown before¹ that duplicate cultures very often give equal results and that there is only slightly more chance for a positive culture from the first point than from the second. One paper point was cultured in brain-heart infusion, the other in brain-heart infusion to which 0.1 per cent of glucose was added. (The commercial product contains a very small amount of glucose.) The order of these two cultures was alternated. The results are given in Fig. 1.

		B.H.I.		
		+	-	
B.H.I. + glucose	+	107	29	136
	-	21	224	245
		128	253	381

Fig. 1.

There seems to be a slight tendency toward B.H.I. plus glucose, which for this small number, of course, is not significant. From the 107 doubly positive cultures, the cultures with glucose showed growth at an earlier time in ten cases. The reverse was true in six cases. There was no obvious difference between the species of organisms cultured on the two media.

Since January, 1958, all primary cultures were made in B.H.I. plus glucose. A definite increase in the frequency of lactobacilli from 2 to 8 per cent was observed after that time. There was possibly also a slight increase in *Streptococcus salivarius* but this may have been due to chance. The increased frequency of lactobacilli is obviously due to the improved growth conditions for this organism in the medium with glucose.

RESULTS

Survey of Flora From All Cultures.—

A total of 4,186 cultures were taken. Of these, 2,989 cultures (72 per cent) were negative and 1,141 (27 per cent) were positive. The frequency distribution of the isolated organisms is given in Table I and Fig. 2. For details, the footnote to Table I should be consulted. In 903 cases one organism was isolated, whereas 238 cultures (21 per cent) contained more than one organism (mixed infections).

TABLE I. STRAINS ISOLATED FROM 4,186 ROOT CANAL CULTURES

ORGANISM	A	B	C	D	E
	IN PURE CULTURE	IN MIXED INFECTIONS		NUMBER OF TEETH	
<i>Streptococcus faecalis</i>	240	45	285	138	2.07
<i>Streptococcus mitis</i>	153	130	283	200	1.42
<i>Streptococcus salivarius</i>	5	15	20	19	1.05
<i>Streptococcus hemolyticus</i>	33	22	55	33	1.67
Anaerobic streptococcus	54	15	69	41	1.69
Indifferent streptococcus	81	71	152	95	1.60
Other streptococci	11	17	28	26	1.08
Total	577	315	892	552	1.62
Micrococci	161	71	232	177	1.32
Lactobacilli	57	41	98	65	1.51
Diphtheroids	45	13	58	48	1.20
Gram-positive rods	10	11	21	15	1.40
Bacillus species	9	12	21	17	1.24
Actinomyces	17	3	20	18	1.11
Fusiformis	5	3	8	7	1.14
Sarcina	1	—	1	1	—
Neisseria	1	14	15	13	1.16
Gram-negative rods	5	23	28	27	1.03
Yeasts	15	8	23	18	1.28
Mixed infection	238				
Total positive cultures	1,141	Total organisms 1,417			
Turbid, no growth	56				
Negative cultures	2,989				
Total	4,186				

In fifty-six cases (1.5 per cent of all cultures) a slight turbidity, which could not be subcultured, was observed.

In column B the frequency of any organism in mixed infections is given. As each mixed infection contributes at least two organisms, the total number of organisms (sum of column C) is appreciably higher than the total number of positive cultures.

Column D gives the number of teeth from which the strains of each group (column C) were isolated. The last column gives the total number of each organism divided by the number of teeth from which they were isolated. This ratio is a measure of the number of times the same organism was isolated from one canal. Organisms with a high ratio seem able to persist longer in the canal. (The ratio was not corrected for the number of teeth with more than one canal.)

The preponderance of streptococci (51 per cent of pure cultures, 63 per cent of all organisms) is obvious. Micrococci, with 14 per cent, and lactobacilli, with 8.5 per cent, are next in number. Neisseria and *Streptococcus salivarius*, though abundant in the mouth, are rare and occur mainly in mixed infections (Table I, column 2), which probably means that they cannot maintain themselves in the root canal. For the same reason, gram-negative rods are considered secondary invaders.

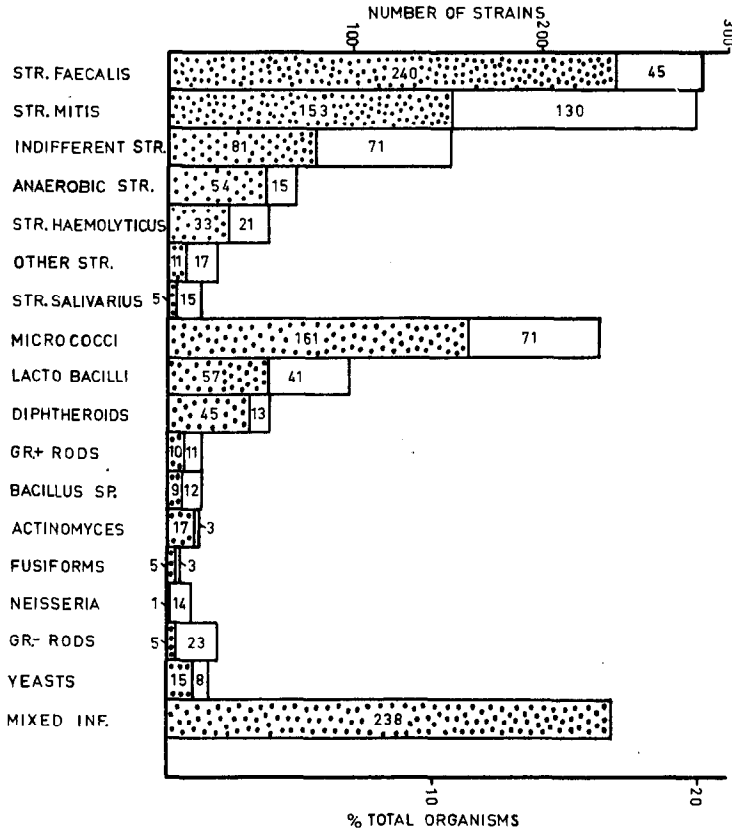


Fig. 2.—Frequency distribution of strains in 1,141 positive cultures. Shaded columns represent strains in pure culture; white columns represent additional strains from mixed infections. The per cent scale at the right refers to all organisms (shaded and white columns together). The shaded columns cannot be referred to this scale (see Table I).

Streptococcus faecalis: All strains presented under this heading showed characteristic large α -hemolytic colonies. All strains withstood heating for 30 minutes at 60° C.; they fermented mannitol and esculin. Most strains were slightly more bile sensitive than the usual fecal strains.¹³ Many strains were grouped serologically; they were always group D.

The strains mentioned as *Streptococcus faecalis* in the table include *Streptococcus liquefaciens*. During a certain period of the study, all strains were classified in this respect. Thus, from July, 1957, to July, 1958, 158 strains of

D streptococci were cultured (144 pure cultures, 14 from mixed infections), from which fifty-nine liquefied gelatin (37 per cent). *Streptococcus faecalis* was often isolated more than once from one canal (mean ratio, 1.89). For *Streptococcus liquefaciens*, this ratio was 2.96, which means that *Streptococcus liquefaciens* persists much longer in root canals than any other organism.

Streptococcus mitis: All α -hemolytic strains that could not otherwise be classified are presented under this heading. None of them produced slime from saccharose on solid medium. All strains that were tested serologically proved ungroupable; some strains were tested with Optochin and proved insensitive. During a certain period, a subdivision by colony form and fermentation reactions was attempted but appeared useless. Twenty randomly selected strains were tested for collagenase by incubating with Azocol¹²; only one strain was positive.

Streptococcus hemolyticus: From the fifty-five strains, forty-nine were grouped serologically. Twenty-eight (57 per cent) were group F, fifteen (30 per cent) were group G, two were group C, and four were not groupable with the available sera. Groups F and G were often isolated more than once from one patient (ratio about 2). For group C and the nongroupable strains, this ratio was 1.

Indifferent streptococci: These strains formed minute colonies on blood agar and did not show any change in the blood medium. All strains were insensitive to Optochin. They generally grew better anaerobically, and consequently the group merged with the next. In the beginning of the study, no classification was attempted. Later, the strains were tested serologically after Fuller extraction. From a series of forty strains, twenty-three reacted with the available sera: group F, ten; group G, six; group C, four; group D, two; and group E, one. Guthof¹⁰ and Wensinek¹⁹ described analogous observations. All groupable strains fermented glucose, lactose, saccharose, maltose, salicin, and trehalose but not raffinose, arabinose, mannitol, sorbitol, or amyllum. Lactose fermentation occasionally was retarded. Five of the ungroupable strains had the same fermentation pattern. The other ungroupable strains showed slightly different patterns. These facts point to a relationship between the groupable indifferent strains and hemolytic strains with the same cell-wall antigen. Ten indifferent strains were subcultured daily for several weeks in blood broth; none became hemolytic. The strains are under study and will be reported upon later. A relationship between these indifferent strains and hemolytic streptococci of groups C, G, and F might be important with respect to problems of focal infection and endocarditis.

Indifferent anaerobic streptococci: None of these strains showed α or β hemolysis; they were completely indifferent toward blood. Judged by the (limited) fermentation tests described by Weinberg and associates,¹⁸ many of these strains could be described as *Streptococcus micros* and some others seemed to belong to *Streptococcus intermedius*. A large number of these strains were groupable. Of a series of ten strains, seven belonged to group C.

The fermentation patterns of these group C streptococci were equal to those of the former group, although three of the anaerobic strains did not ferment trehalose.

Although the evidence is incomplete, there seem to be many indications that the hemolytic, indifferent, and indifferent anaerobic streptococci isolated here are in some way related. That there exist other reasons to consider them as one group will be shown below.

Other streptococci: All the strains mentioned under this heading in Table I were α hemolytic. Some differed in colony form from the usual *Streptococcus mitis*. Some strains were scored as *Streptococcus mitis* and lost before further study; others remained completely unidentified. The whole group should probably be added to the mitis category.

Lactobacilli: Lactobacilli were rare (2 per cent of all organisms) during the first part of the investigation. Following the introduction of brain-heart infusion with glucose as primary culture medium, their number increased (8 per cent). Judging from the colony form on Rogosa medium, the strains were mainly *Lactobacillus casei* (or *plantarum*). Twelve strains were further identified. Of those, nine were *Lactobacillus casei*, one was *L. fermenti*, and one was *L. plantarum*.

Mixed infections: Of 196 mixed infections, 173 contained two organisms, and 20 contained three organisms; four organisms were cultured twice, and once five organisms were cultured. Among the double combinations *Str. faecalis* was found thirty-one times, *Str. mitis* 100 times, indifferent streptococci twenty-three times, micrococci eight times, and *Str. hemolyticus* ten times. Among the triple combinations, *Str. faecalis* was present eight times, and *Str. mitis* was present ten times.

Micrococci: Twice, a coagulase positive *Micrococcus pyogenes* was isolated. All other isolated strains showed white colonies on blood agar. All were an-hemolytic and coagulase-negative. It will be argued below that the bulk of these strains are due to chance contaminations.

Bacterial Flora From Different Clinical Situations.—

There were complete case histories from 902 patients (canals). These involved 3,312 cultures, of which 993 (30 per cent) were positive. A slight turbidity in the primary culture which could not be subcultured (T.N.G. = turbid, no growth)* was present in thirty-six cases. There were 2,317 (70 per cent) negative cultures.

The complete data, differentiated for vital pulps, open or closed necrotic pulps, and old endodontic cases, are given in Table IIA for initial cultures and in Table IIB for later cultures. Some details are discussed in the footnote to these tables. In Table III a summary is given, and it is convenient to discuss this table only.

*Possibly abundant blood from pulp.

TABLE IIIA. FREQUENCY OF ORGANISMS IN INITIAL CULTURES

ORGANISM	VITAL PULPS			OLD CANAL TREATMENTS			OPEN NECROTIC PULPS			CLOSED NECROTIC PULPS		
	P	T	PER CENT	P	T	PER CENT	P	T	PER CENT	P	T	PER CENT
1. <i>Streptococcus faecalis</i>	35	7	15	11	13	6	3	6	8	18	25	19
2. <i>Streptococcus mitis</i>	42	68	29	23	29	10	21	29	21	21	37	28
3. <i>Streptococcus salivarius</i>	1	2	1	0	2	2	0	5	7	1	1	1
4. <i>Streptococcus hemolyticus</i>	2	3	1	0	1	1	1	5	7	2	4	3
5. Anaerobic streptococci	2	5	2	5	6	0	1	1	3	3	3	2
6. Indifferent streptococci	6	15	6	5	9	11	2	8	11	5	16	12
7. (4 + 5 + 6)	23	10	15	18	18	14	14	19	19	23	23	17
8. Total streptococci	128	55	51	62	62	46	64	64	64	86	86	65
9. Micrococci	22	39	17	12	14	7	2	10	7	7	12	9
10. Lactobacilli	9	12	5	8	9	0	0	6	8	3	8	6
11. Diptheroids	17	22	9	4	5	2	4	6	6	6	9	7
12. Bacillus species	1	3	1	0	3	-	-	3	1	1	3	2
13. Actinomyces	1	1	-	-	-	1	3	5	2	2	2	1
14. Fusiformis	-	0	-	-	-	-	-	-	-	-	-	1
15. Neisseria	0	5	2	-	-	0	2	-	-	-	-	1
16. Gram-negative rods	7	10	4	1	4	5	0	2	0	0	2	1
17. Yeasts	13	13	6	1	1	1	1	1	1	0	1	1
18. T.N.G.	13	13	6	-	-	1	1	1	1	7	7	5
19. Subtotal	150	233	100	49	83	23	72	72	80	132	132	100
20. Mixed infections	42	15	9	8	8	16	8	36	31	31	31	14
21. Total positive cultures	192	41	64	38	39	89	111	89	111	111	111	51
22. Negative cultures	279	59	105	62	5	11	5	11	107	107	107	49
23. Total cultures	471	100	169	62	44	44	44	44	218	218	218	100

In each column the first figure refers to the number of strains present in pure culture (P); the second gives the total number of times strains of that denomination were isolated (T). The number of strains occurring in mixed infections is equal to the difference between T and P. The last figure in each column gives the frequency of the "spreader" as a percentage of the total number (line 19). Below the double horizontal line, percentages are given in terms of the total number of cultures.

The results for all necrotic pulps (the sum of columns 2, 3, and 4) are given in Table III. The number of cases of old canal treatments and open necrotic pulps is small. In some cases it is hardly enough to permit calculation of percentages.

In old canal treatments anaerobic streptococci are high in initial cultures (Table IIIA, line 5), but this is not true for later cultures (Table IIB, line 5).

In open necrotic pulps *Streptococcus faecalis* is infrequent (line 1, both tables). Hemolytic streptococci are frequent (line 4, both tables). The whole group of anaerobic, indifferent, and hemolytic streptococci in later cultures is very frequent (Table IIB, line 7). These organisms seem the best settlers compared with the rest of the oral flora.

TABLE IIB. FREQUENCY OF ORGANISMS IN LATER CULTURES

ORGANISM	VITAL PULPS			OLD CANAL TREATMENTS			OPEN NECROTIC PULPS			CLOSED NECROTIC PULPS		
	P	T	PER CENT	P	T	PER CENT	P	T	PER CENT	P	T	PER CENT
1. <i>Streptococcus faecalis</i>	53	62	21	24	24	28	4	5	9	53	58	23
2. <i>Streptococcus mitis</i>	29	56	19	5	9	10	8	13	22	30	51	20
3. <i>Streptococcus salivarius</i>	2	6	2							1	2	0.8
4. <i>Streptococcus hemolyticus</i>	1	4	1	1	1	1	7	13	22	13	13	5
5. Anaerobic streptococci	9	10	3	1	3	3	2	4	7	20	23	9
6. Indifferent streptococci	21	31	11	8	11	13	9	11	19	9	15	6
7. 4 + 5 + 6	45	45	15	15	15	17	28	28	48	51	51	20
8. Total streptococci	169	169	58	48	48	55	36	36	62	162	162	64
9. Micrococci	52	72	25	15	16	18	4	4	7	30	39	15
10. Lactobacilli	8	16	5.5	10	12	14	0	3	5	6	13	5
11. Diptheroids	7	11	4	3	4					8	10	4
12. Bacillus species	5	6	2	0	1					2	4	2
13. Actinomycetes	2	2	1	1	1					4	4	2
14. Fusiformis										2	6	2
15. Neisseria				0	2					0	4	2
16. Gram-negative rods	1	9	3	1	2		0	1	2	2	2	1
17. Yeast	6	9	3	1	1		2	2	2	1	1	1
18. T.N.G.	3	3	1	1	1		2	2	2	9	9	3
19. Subtotal	198	289	70	87	87	38	58	58	190	254	254	
20. Mixed infections	43		4	8	8	2	8	8	6	32	32	5
21. Total positive cultures	241		20	78	78	18	46	46	32	222	222	34
22. Negative cultures	934		80	348	348	82	98	98	68	441	441	66
23. Total cultures	1,175		100	426	426	144	144	144	663	663	663	
24. Patients (canals)	471		169	169	169	44	44	44	218	218	218	

TABLE III

ORGANISM	INITIAL CULTURES						NOT INITIAL CULTURES						ALL CULTURES						TOTAL	
	I (VITAL)		II (NECROTIC)		III (VITAL)		IV (NECROTIC)		V (VITAL)		VI (NECROTIC)		VII (INITIAL)		VIII (NOT INITIAL)		NUM-BER	PER CENT		
	NUM-BER	PER CENT	NUM-BER	PER CENT	NUM-BER	PER CENT	NUM-BER	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT					
1. <i>Streptococcus faecalis</i>	35	15	42	15	62	21	22	87	22	19	19	15	22	22	226	19				
2. <i>Streptococcus mitis</i>	68	29	81	28	56	19	18	73	18	24	22	29	19	278	23					
3. <i>Streptococcus salivarius</i>	2	1	8	3	6	2	0.5	2	2	1	1	2	1	18	1					
4. <i>Streptococcus hemolyticus</i>	3	1	10	3	4	1	7	27	7	1	5	2	4	44	4					
5. Anaerobic streptococci	5	2	9	3	10	3	7	30	7	3	6	3	6	54	4					
6. Indifferent streptococci	15	6	33	11	31	11	9	37	9	9	10	9	10	116	10					
7. 4 + 5 + 6	23	10	52	18	45	15	24	94	24	13	21	14	20	214	18					
8. Streptococci total	128	55	183	64	169	58	64	256	64	57	64	60	61	736	61					
9. Micrococci	39	17	31	11	72	25	15	59	15	21	13	13	19	201	17					
10. Lactobacilli	12	5	22	7	16	5	7	28	7	5	7	6	6	78	6					
11. Diphtheroids	22	9	17	6	11	4	4	16	4	6	5	7	4	66	5					
12. Other organisms	19	8	26	9	18	6	7	28	7	7	8	9	7	91	7					
13. T.N.G.	13	6	8	3	3	1	3	12	3	3	3	4	2	36	3					
14. Total	233	100	287	100	289	100	399	100	-	-	-	-	-	1,208	100					
15. Cultures with streptococci	116	25	151	35	154	13	235	19	16	23	30	30	16	656	20					
16. Cultures with micrococci	39	8	31	7	72	6	59	5	7	5	8	8	5	201	6					
17. Total positive cultures	192	41	214	50	241	20	346	28	26	34	45	45	24	983	30					
18. Negative cultures	279	59	217	50	934	79	887	72	74	66	55	55	76	2,317	70					
19. Total cultures	471		431		1,175		1,233							3,310						

In each column the first row gives the number of strains of the indicated "species" which were isolated. The second row gives the frequency of each organism as a percentage of the total number of organisms, including T.N.G. (line 14). In columns V to VIII the first row is omitted. The omitted numbers are the sums of the numbers in columns I + III, II + IV, I + II, and III + IV, respectively.

Line 8 gives the frequency of all streptococci; line 7 gives the frequency of the hemolytic, indifferent, and indifferant anaerobic streptococci, which in several respects can be considered as one group.

Below the double horizontal line the percentages are given in terms of the total number of cultures (line 19).

The last column (IX) lists the frequency of each organism as percentage of the total number of organisms isolated. *Streptococcus faecalis* was present in 19 per cent, *Streptococcus mitis* in 23 per cent, and micrococci were present in 17 per cent. There is again a definite preponderance of streptococci, which form 61 per cent of all organisms (line 8) and occur in 20 per cent of all cultures (line 15).

Comparing initial cultures with later cultures, we find that the percentage of positive cultures is much higher among the initial cultures (45 per cent, column VII, line 17) than among later cultures (24.3 per cent, column VIII). As the cultures were taken at different stages of preparation and from all canals until one negative culture had been obtained (including one control culture immediately before filling which generally was negative¹), this preponderance of negatives among later cultures is obvious.

If one is to understand the meaning of the frequency distribution of different organisms in the cultures, some theoretical considerations seem necessary.

The origin of the cultured bacteria is obviously of different kinds:

1. If we consider the procedure for taking initial cultures from accidentally exposed sound pulps, it is obvious that even with the strictest technique the chances of carrying bacteria of the carious cavity into the pulp during perforation of the roof of the pulp chamber are large. Contamination with salivary organisms through leaky rubber-dams should also be considered. This means that the number of positive cultures from pulps that did not contain bacteria before preparation will be large. In these cases the number of bacteria will probably be relatively small. As for the kind of organisms one may expect, all organisms that can be present in the deep carious lesion. If the mechanical preparation of the root canal does not immediately follow exposure of the pulp, some of these organisms might establish a clinical infection.

2. In the case of actual infection of the root canal, as in pulpitis and most cases involving necrotic pulps, the causal organism will nearly always have penetrated into the pulp from the carious lesion. Not all organisms will be able to maintain themselves in the pulp; only a small fraction of the caries flora will be able to overcome the natural defense of the living pulp. In infected and in necrotic pulps one may consequently expect a much smaller number of species, but the species that establish themselves will be present in relatively large numbers (unless the lesion has "sterilized" itself).

3. Finally, the chance contamination of the culture from the air, from instruments, or from the hands of the operator indirectly is to be considered.

Differences Between Flora From Vital and Necrotic Pulps.—From the difference between the flora from vital and necrotic pulps, it would seem easy to

decide which organisms were possible pathogens and which were chance contaminants. The difficulty is that the chance contamination during the opening of the canal is superimposed on the flora of the infected canal. Only quantitative evaluation of the number of bacteria of different species in the sample would permit a distinction between the cases mentioned under 1 and 2 above. With the present technique, any chance contaminant will grow in the fluid primary culture and confuse the issue.

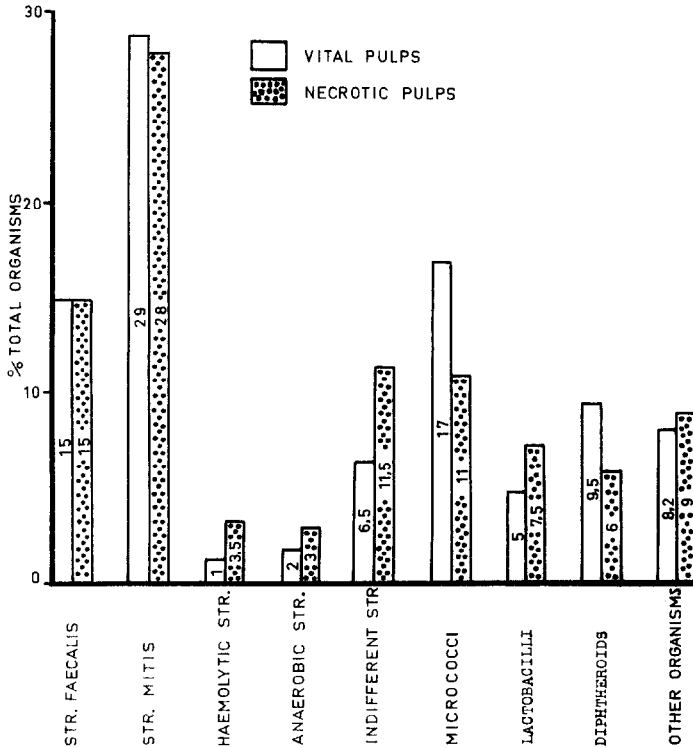


Fig. 3.—Frequency distribution of strains in initial cultures. The hemolytic, anaerobic, and indifferent streptococci and the lactobacilli occur with increased frequency in necrotic pulps. All other organisms are less frequent in necrotic than in vital pulps or show equal frequencies.

Nevertheless, some indications concerning an eventual pathogenic significance of some species might be obtained by comparing cultures from exposed sound pulps with those from necrotic pulps (Table III). On comparison of columns I and II, III and IV, and V and VI (line 17), it can be seen that the percentage of positive cultures from necrotic pulps is higher (41 to 50 per cent for initial cultures, $p < 0.05$, for the other differences $p < 0.01$). No large differences in the flora seem to exist. However, there is a definite increase in the percentage of streptococci (line 8), mainly due to the groups of hemolytic, anaerobic, and indifferent streptococci (Table III, line 7; 10 to 18 per cent for initial, 15 to 24 per cent for later, and 13 to 21 per cent for all cultures, all

$p < 0.05$; compare also Figs. 3 and 4). This seems to point to a possible role for these organisms as primary invaders. *Streptococcus mitis* is equally frequent in all cases.

The group of micrococci appears to be found in a fairly constant percentage of all cultures in all groups (Table III, line 16; only the difference between columns VII and VIII has a $p < 0.05$). If calculated as percentage of all organisms (line 9), the micrococci are less frequent in cultures from necrotic pulps (21 \rightarrow 13 per cent columns V and VI; $p < 0.01$). This is obviously due to the higher percentage of positive cultures from necrotic pulps. They are also less frequent in initial cultures than in later cultures (line 9, columns VII and VIII). This, again, is due to the higher frequency of negatives among the

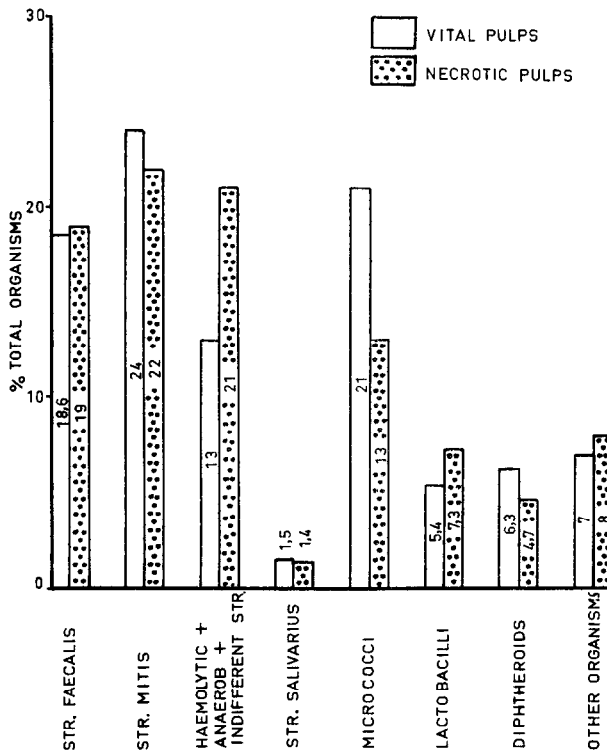


Fig. 4.—Frequency distribution of strains in all cultures. The group of hemolytic, anaerobic, and indifferent streptococci occurs with increased frequency in cultures from necrotic pulps.

later cultures. This seems to indicate that they are mainly chance contaminants. This view is supported by the fact that only coagulase-negative micrococci (abundant on human skin) were found. The point is further stressed by the much lower percentage of staphylococci found in cultures taken by experienced dentists than in those taken by students (Table IV).

Organisms Persisting in the Root Canal.—In comparing the flora of initial cultures with that of later cultures, one might acquire some information about

TABLE IV. FREQUENCY OF MICROCOCCI IN CULTURES FROM EXPERIENCED DENTISTS AND STUDENTS

CULTURE	DENTISTS		STUDENTS	
	NUMBER	PER CENT	NUMBER	PER CENT
Cultures with micrococci	17	2.5	184	7
Cultures with streptococci	133	20	523	20
Cultures with other bacteria	26	4	144	5.4
Positive cultures	172	26	821	31
Negative cultures	493	74	1,824	69
Total	665		2,645	
Micrococci (per cent from positive cultures)		10		22

The sum of the cultures with micrococci, streptococci, and other bacteria is not equal to the number of positive cultures, as there were four mixed infections with micrococci and streptococci in the dentist group and twenty in the student group.

The most experienced dentist had 1.8 per cent cultures with micrococci; the other 3.5 per cent.

the organisms that are able to persist in the root canal. On comparison of columns I and III, II and IV, and VII and VIII (Table III), there seem to be no large differences. This is due again to the large background of chance contaminations. Studied more closely, however, it appears that *Streptococcus faecalis* is present more frequently in later cultures (15 to 22 per cent, line 1, columns VII and VIII). The same is true for hemolytic, anaerobic, and indifferent streptococci (14 → 20 per cent, line 7). *Streptococcus mitis* is less

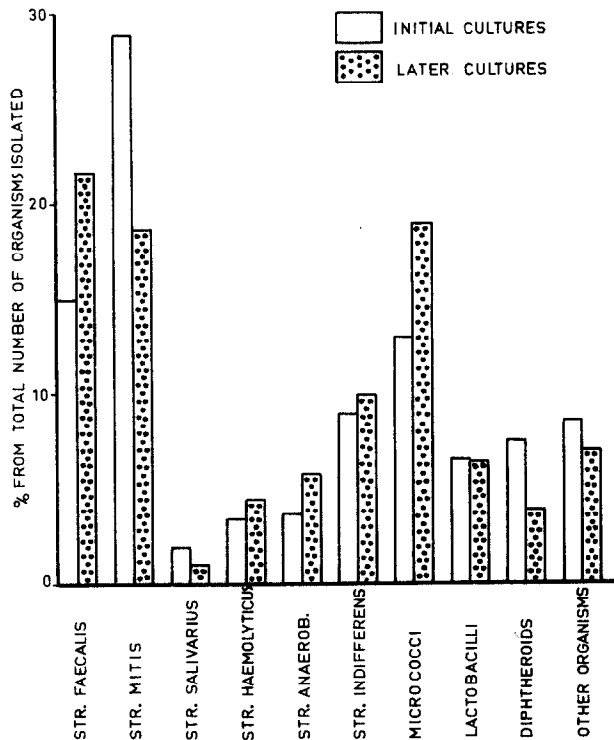


Fig. 5.—Frequency distribution of strains in all cultures. Organisms that persist in the root canal will be present with higher frequency in later cultures. These are *Streptococcus faecalis*, the hemolytic, anaerobic, and indifferent streptococci. The apparent increase of micrococci is due to other causes (see text).

frequent in later cultures. *Streptococcus faecalis*, *Streptococcus hemolyticus*, and the anaerobic streptococci seem to persist better in the root canal than do other organisms (Fig. 5).

Another approach to the possible role of the isolated strains in root canal infection is the study of cases in which the same organism was isolated more than once from the same canal. This was performed by counting the cases in which the organism found in the first culture was again found (consecutively) in the second, third, etc., cultures from the same canal. In the cultures from vital pulps there were forty-three (24 per cent) such cases where an organism was found more than once. In the group from necrotic pulps this number was eighty (39 per cent). The distribution of organisms is given in Table V.

In some cases the numbers seem small. It should be remembered, however, that the probability for a consecutive series of three cultures of, for example, *Streptococcus hemolyticus* is extremely small (compare note under Table V). It is obvious that some organisms persist whereas others do not (Table V, last three columns). The organisms that persist longest (that is, maintain themselves best) seem to be *Streptococcus hemolyticus*, *Streptococcus faecalis*, indifferent streptococci, indifferent anaerobic streptococci, and perhaps lactobacilli and *Streptococcus mitis*. All other organisms are present only in the initial cultures, and as they disappear after preparation they do not seem to have settled themselves in the dentine or in the periapical tissues. The separate results for vital and necrotic pulps are given in the first half of Table V. Long series of consecutive cultures occur mainly in the group with necrotic pulps. For *Streptococcus faecalis*, *Streptococcus hemolyticus*, the indifferent streptococci, and lactobacilli, the percentage of consecutive positives is higher in the cultures from necrotic pulps. This is not so for micrococci and *Streptococcus mitis*; consequently, we consider these organisms as less important.

In many cases given as consecutive series of cultures of *Streptococcus faecalis*, it was definitely *Streptococcus liquefaciens* which was isolated. If we divide the number of times *Streptococcus liquefaciens* was isolated by the number of all canals in which *Streptococcus liquefaciens* was found, the ratio is 3, as has already been discussed. As the ratio for *Streptococcus faecalis* is only 2, there is no doubt that *Streptococcus liquefaciens* persists better in the root canal than *Streptococcus faecalis*.

The fact that long series of consecutive cultures positive for one organism can occasionally be obtained from vital pulps requires explanation. Apart from the obvious difficulty in clinical diagnosis, we think that in some of these cases a contamination during opening of the canal could have established itself as a clinical infection in the period between instrumental entry and mechanical preparation (generally one week).

In an analogous calculation the cases with more than one culture with the same organism were counted but the requirement of consecutiveness was omitted. The results pointed in the same direction. The number of cases was somewhat larger. The order of the organisms, in terms of frequency of three or more

TABLE V. NUMBER OF CANALS SHOWING THE SAME ORGANISM MORE THAN ONCE CONSECUTIVELY

ORGANISM	VITAL PULPS						NECROTIC PULPS						VITAL + NECROTIC					
	+	-	-	++	+++	++++	TOTAL	PER CENT	++ OR MORE	+	-	-	++	+++	++++	TOTAL	PER CENT	++ OR MORE
<i>Streptococcus faecalis</i>	17	10	(2)	+	+	+	31	45								69	58	27
<i>Streptococcus hemolyticus</i>	2						2	0								10	60	40
Anaerobic streptococci	9	3	(0.5)	1	1	1	13	(60)								16	56	25
Indifferent streptococci	45	12	(3)	3	—	—	60	25								38	50	21
<i>Streptococcus mitis</i>	24	4	(2.3)	1	—	—	29	17								118	29	6
Micrococci	16			—	—	—	16	0								52	17	4
Diphtheroids	9			1	—	—	10	10								33	6	6
Lactobacilli	11	1		—	—	—	12	8								22	36	13
Others	135	31		6	6	6	178	24								382	33	13
Total									124	43	22	15	204	39				

*The numbers in parentheses in the columns +- refer to the frequency for two consecutive positive cultures expected from chance alone.

positive cultures from one patient, was again *Streptococcus faecalis*, *Streptococcus hemolyticus*, *anaerobic streptococci*, *indifferent streptococci*, lactobacilli, *Streptococcus mitis* and micrococci.

DISCUSSION

The chance of carrying bacteria from the carious cavity into the root canal during the opening of the canal is relatively great. Consequently, a large fraction of root canal cultures, even those from exposed sound pulps, will be positive due to this type of contamination. The large background of chance contamination hampers the analysis of results. This large background is due partly to the fact that most cultures discussed in this article were taken by relatively inexperienced operators. The study of an equally large amount of material gathered by experienced dentists would be much more useful.

Another serious difficulty in the interpretation of results is the fact that the primary culture had to be made in fluid medium, in which some organisms will have been selected and others suppressed. Development of a method for a more quantitative evaluation of the root canal flora is obviously of great importance. Until such a method has been developed, it will be difficult to arrive at definite conclusions concerning the pathogenic importance of the isolated organisms.

Recognizing these limitations, however, we believe that an analysis of the available results permits some tentative conclusions.

The coagulase-negative micrococci, which form 17 per cent of all organisms, are to be considered as chance contaminants. Irrespective of the conditions of the canal or the stage of treatment, they occur with equal frequency in all cultures. Expressed as a fraction of total organisms, they are less frequent in infected pulps than in sound pulps. The number of two consecutive cultures with micrococci from one canal does not differ greatly from the number expected from chance alone (Table V). Furthermore, there is a definite negative correlation between the frequency of micrococci and the technical skill of the operator (Table IV).

For *Streptococcus mitis* the data are much less definite. This organism might also be a chance contaminant, as it occurs with equal frequency in cultures from vital and from necrotic pulps and is less frequent in later cultures (Table III). The number of consecutive cultures with *Streptococcus mitis* from one canal is equal for vital and for necrotic pulps (Table VI) and, although it is larger than from chance alone, the difference is much less than for any other organism (except micrococci). As the group called *Streptococcus mitis* is probably heterogeneous, it is quite possible that it still contains a fraction of possible pathogens. A large fraction are probably chance contaminants, however, as was to be expected from the organism's abundance in the mouth.

Among the other streptococci, the hemolytic indifferent and anaerobic indifferent streptococci seem to be serious candidates for a possible role as the cause of pulp infections. The same seems to be true for the group D streptococci, although the evidence is less unequivocal here.

The hemolytic, indifferent, and anaerobic indifferent streptococci can be treated as one group because, irrespective of the differences in oxygen requirement and hemolysis, they show relationships in cell-wall antigens and fermentation patterns. In the future, it would be better perhaps to give priority to the serologic character and discuss the significance of F, G, and C streptococci separately. At the moment, we will take the whole group together.

As they are more frequent in cultures from necrotic pulps than from vital pulps (Figs. 2 and 3), more frequent in later than in first cultures, have a high mean ratio of cultures per canal (Table I), and occur more often in consecutive series of positive cultures from one canal, especially in necrotic pulps (Table V), these streptococci can be considered as possible pathogens. The fact that nonhemolytic streptococci seem to act as possible pathogens might be of importance from the point of view of focal infection and endocarditis.

The D streptococci persist tenaciously in root canals, as can be seen from their higher frequency in later cultures (Table III), from the high ratio of organisms per canal (Table I), and from the long series of consecutive cultures from one canal (Table V). *Streptococcus liquefaciens* is even more persistent in this respect than *Streptococcus faecalis*. D streptococci are difficult to eliminate from the root canal.

This persistence seems to point to a possible role as a pathogen. One would then expect a higher frequency in cultures from infected pulps than from vital pulps. The observed difference, however, is negligible (Fig. 4) or absent (Fig. 3). It should be remembered that the frequency of chance contaminants (for example, micrococci and diphtheroids) is definitely lower in cultures from necrotic pulps (Fig. 4). The D streptococci take an intermediate position between the chance contaminants which occur less frequently in cultures from necrotic pulps and the organisms which are considered possible pathogens because they are more frequent in cultures from necrotic pulps. We think that the expected higher frequency of D streptococci in cultures from necrotic pulp is obscured by the large chance of contamination by this organism. The high frequency of the number of series of consecutive positive cultures of D streptococci from necrotic pulps (68 per cent) compared with the frequency from vital pulps (45 per cent, Table V) supports the view that D streptococci are possible pathogens.

These results seem to point to a large chance of contamination with D streptococci. In some cases they seem to be able to cause clinical infections, and if they do they are difficult to eliminate. For these reasons, we think that they should be considered potential pathogens.

Lactobacilli seem also to be more frequent in necrotic pulps and are occasionally able to persist (Table V). Without further data, no conclusion is warranted since the numbers are too small.

SUMMARY

The bacteriologic results from more than 4,000 root canal cultures are discussed. Streptococci form 61 per cent of the isolated organisms. Among these,

the group of hemolytic, indifferent, and indifferent anaerobic streptococci, mainly belonging to serologic groups F, G, and C, seem the most serious possible pathogens. D streptococci (especially *Streptococcus liquefaciens*) are difficult to eliminate from the root canal and are also potential pathogens. *Streptococcus mitis* seems of much less importance, and all other organisms are to be considered as chance contaminants.

Many persons from the staff of the Laboratory have helped with the isolation and identification of organisms or with the calculation of results. Special thanks are due Mr. R. Braakenburg v. Backum, Mr. H. Ottens, Miss H. G. S. Vreede, Miss W. I. A. v. Thienen, and Miss M. H. G. Tressel.

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