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THE BACTERIOLOGICAL CONTROL OF ENDODONTIC TREATMENT

III. Bacteriological examination of root canals, treated with polyantibiotics and other medicaments

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Introduction

The use of polyantibiotics in endodontia is based essentially on the investigations of GROSSMAN (1, 2, 3, 4) and SELTZER and BENDER (5, 6). The results indicate that a very small number of treatments – averaging from 1,1 to 1,4 – is required to obtain sterility of the root canal. In the investigations cited, a canal was considered sterile if it yielded negative cultures. This assumption is subject to the following critisism.

When taking a culture from a root canal, treated with a desinfectant, residues adhering to the absorbent point may inhibit growth, resulting in a false negative culture.

To prevent this the above mentioned authors recommend, before taking a culture, to remove every trace of the medicament by wiping the canal thoroughly two or three times with an absorbent point. BUCHBINDER and BARTELS (7) made growth inhibition tests from root canals, treated with polyantibiotics, which had been cleaned in this way. All cultures showed inhibition of growth on agar plates, inoculated with Escherichia coli and Staphylococcus aureus, or even after dilution in liquid culture media. Consequently they consider cultures from root canals, treated with polyantibiotics, unreliable unless inactivators are used against the components of the medicament.

In a similar study, BENDER and SELTZER (8) used a liquid culture medium, commonly used for culturing material from root canals. They proceeded as follows: 46 root canals, from which negative cultures had been obtained, were filled with polyantibiotic paste and an absorbent point. On return of the patient the absorbent point was removed and the canal was wiped with three points in succession. The resulting four points from each case were dropped into four culture tubes, containing 10 ml Brain

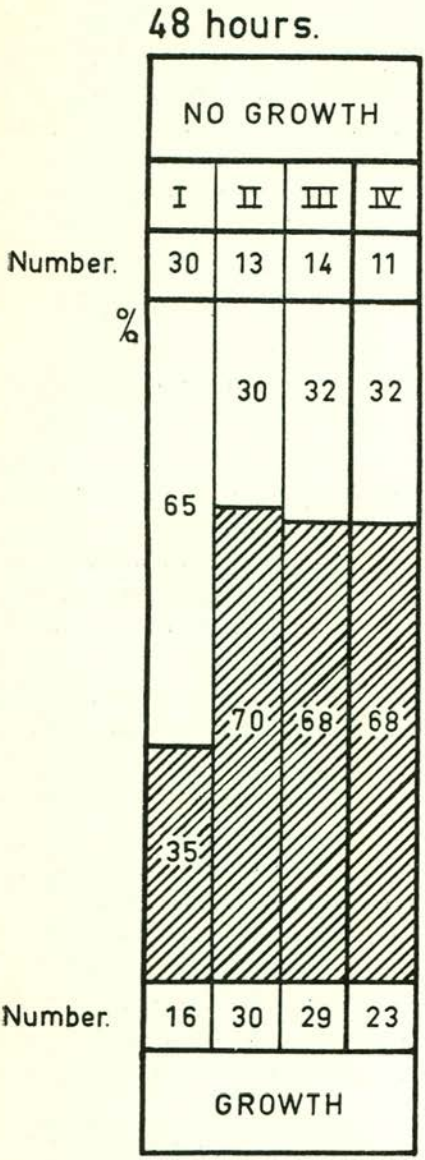

Fig. 1

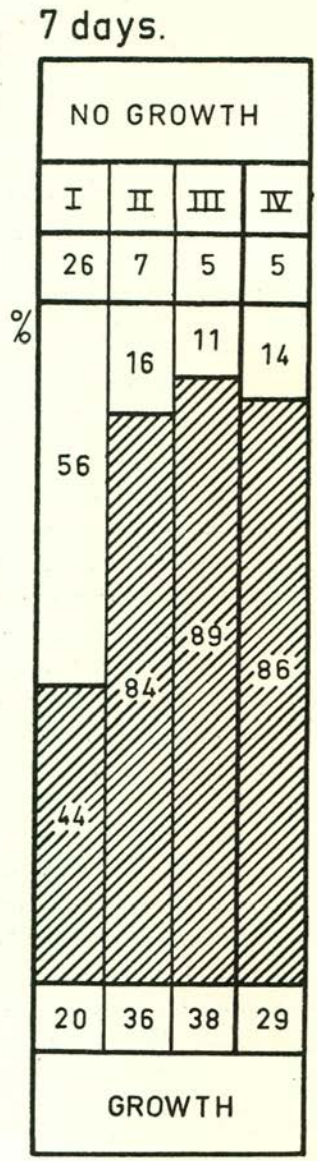

Fig. 2

Growth inhibition in cultures from root canals treated with antibiotics (From I. B. Bender and S. Seltzer[8])

heart infusion broth. Subsequently, these culture media were inoculated with an inoculum, obtained from infected root canals, which had been mechanically cleansed to rid them of gross debris. Diagrams 1 and 2 show the results after incubation during 48 hours and 7 days respectively. Bars I, II, III and IV show the growth – growth inhibition ratio in each of the four culture tubes; the shaded part of the bars indicates the percentage of growth.

From these data one may conclude:

1. The first absorbent points, obviously saturated with polyantibiotic paste, cause a growth inhibition in 65% and 56% respectively. (Bar I)
2. The subsequent absorbent points show less growth inhibition but the difference between the three is small viz. in 30% and 16%, (bar II), 32% and 11% (bar III) and 32% and 14% respectively (bar IV).

These results are unexpected, because the growth inhibition does not decrease with the decreasing quantities (III and IV) of the antibiotics. As the inocula seem to have been inhomogeneous and inconstant the results are difficult to interprete.

It is not likely that these experiments are very close to reality because in practice we take cultures from canals which have been submitted to a thorough mechanical preparation and moreover have been medicated. We must therefore take into consideration that a very small number of bacteria may be present, and consequently the number of cases with growth inhibition will be even greater than the diagram suggests.

It is clear that ways have to be found to prevent these growth inhibitions. BENDER and SELTZER (8), like BUCHBINDER and BARTELS (7), require that this should be done by means of inactivators.

Here, however, two difficulties arise:

1. against some of the antibiotics used, no inactivators are known.
2. some inactivators are not stable and therefore not suitable for practical use.

The question arises whether the appearance of false negative cultures could not be eliminated in an easier way, e.g. by a more efficient way of cleaning the medicated root canals prior to taking the culture. The solution of this question was the aim of this study. It seemed important to choose a set-up in which growth inhibition can easily be assessed. This can be achieved by:

1. inoculating the culture medium with the smallest possible inoculum that will give consistent growth.
2. by selecting a species of microorganisms that is very sensitive to antibiotics.

Experiments

First, a growth inhibition test was made immediately after mechanical preparation and irrigation with chlorinated soda and hydrogen peroxide, a method advocated among others by GROSSMAN (1).

It is conceivable that cultures taken at this stage might produce false negative cultures due to growth inhibition by the chemicals. (STEWART 9).

In 25 cases, root canals, irrigated with these medicaments, were dried with 4 absorbent points, each of which was separately deposited in a culture tube, containing 5 m.l. Brain heart infusion broth (Difco), closed with sterile cotton. Within two hours all tubes were inoculated each with one „10–4" drop of an overnight culture of penicillin-sensitive staphylococci. The use of a more diluted suspension is not possible since in that case the chance exists that no growth will occur. The culture tubes were incubated during 72 hours at 37° C. Incubation for a longer period of time was not considered necessary since it appeared that no more growth would occur.

Another test was made, in which 50 root canals, after mechanical preparation, were filled with camphorated chlorphenol (ChKM, Walkhoff) on an absorbent point. After application of a sterile cotton pellet to the entrance of the root canal, the cavity was sealed with zinkoxide-eugenol cement and gutta percha during 7 days.

After removal of the temporary filling materials and the cotton pellet, the dressing was placed in a culture tube. Two absorbent points, used for wiping and drying the canal were processed in the same way. Inoculation and incubation was carried out as described.

Finally a number of 129 root canals were filled, after mechanical preparation, with a polyantibiotic paste of the following composition:

Dihydrostreptomycine	200 mg
Chloramphenicol	200 mg
Potassium-penicillin G	200.000 I.E.
Sodium caprylate	200 mg
Silicone fluid . . . . . .ad	1,6 g

The canal was sealed as mentioned before.

At the next visit (after one week), after removal of the temporary filling

materials and the cotton pellet, the canal was cleaned from paste by thoroughly wiping with three successive absorbent points. Generally on the last point no paste can be observed macroscopically. These three point were deposited in three culture tubes, numbered consecutively I, II and III.

In addition, 52 canals out of the 129 were again wiped twice with absorbent points, which were placed in culture tubes numbered IV A and V A. These last two points would usually be considered suitable for making cultures.

For comparison, in the remaining 77 cases an attempt was made to remove traces of paste, possibly remaining in the apical end of the canal, by means of a thin Hedström file, after which the canal was irrigated with 2 m.l. sterile saline solution. After drying, the canal again was wiped with two absorbent points which in turn were placed in culture tubes numbered IV B and V B. As a measure of control, in all 129 cases a sterile point was deposited in a sixth culture tube (numbered VI).

Again, all culture tubes were inoculated and incubated as described.

Results

Experiments in vitro, in which absorbent points were saturated with chlorinated soda, did not show any growth inhibition. From the 25 root canals irrigated with chlorinated soda and hydrogen peroxide alternately, none of the 4 successive absorbent points, used in each case to dry the canals, showed growth inhibition.

Preliminary growth inhibition tests in vitro with camphorated chlorphenol, even in very small amounts, in the clinical sense, showed growth inhibition in all cases. The result was different however, if the medicament had remained during one week in a sealed root canal. In a series of 50 cases it appeared that a ChKM-saturated absorbent point, after one week's stay in a sealed root canal, showed growth inhibition in only 3 cases (6%). Another point, used for wiping the canal after the dressing had been removed, showed growth inhibition in no instance, nor did a next point, used in the same way.

The diagrams 3 and 4 show the results of the tests, in which polyantibiotics were used, after incubation during 24 and 72 hours respectively. The bars numbered I to VI show the growth – growth inhibition ratio; the shaded part of each bar and the corresponding figure indicate the percentage of growth.

The bars numbered I, for instance, show that after 24 hours incubation one culture (1%) yielded growth and 128 cultures (99%) showed growth inhibition. After 72 hours these numbers were 4 and 125 (3% and 97%)

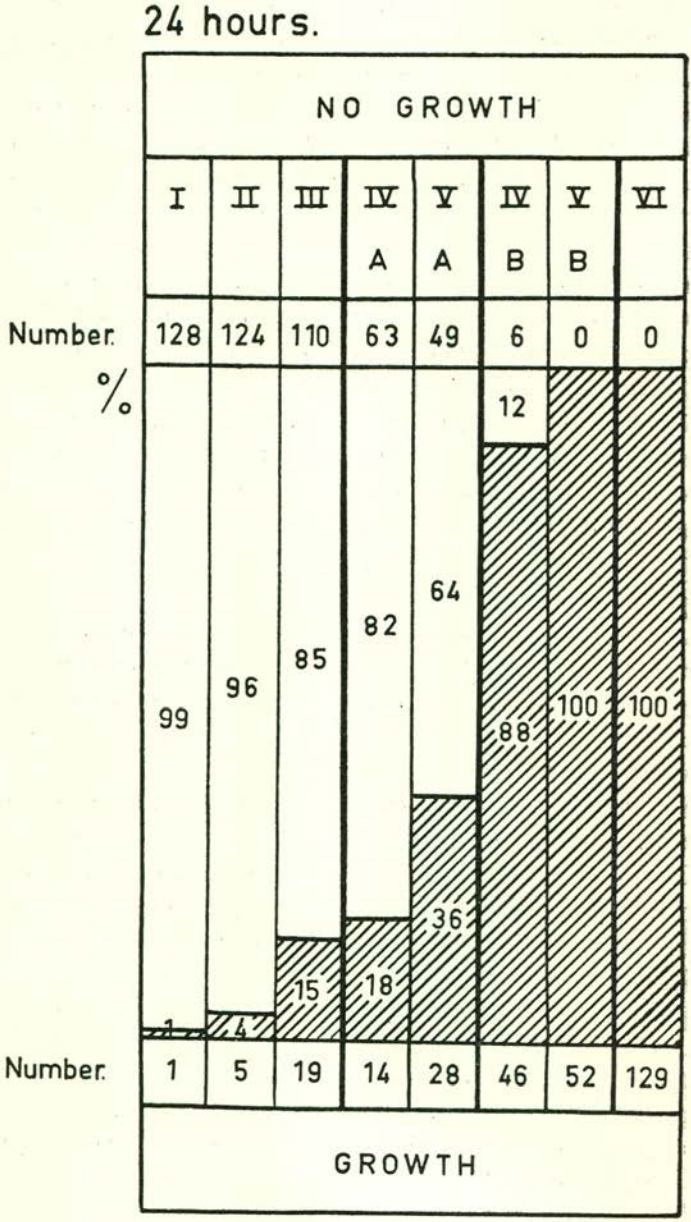

Fig. 3

Growth inhibition in cultures from root canals treated with antibiotics.

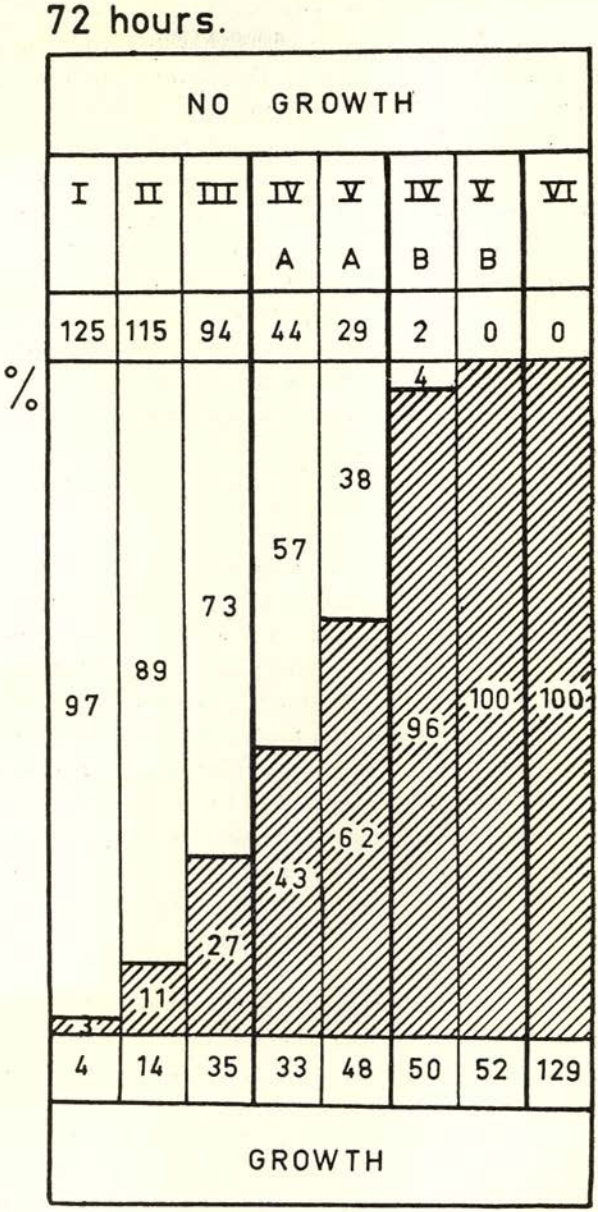

Fig. 4

Growth inhibition in cultures from root canals treated with antibiotics

respectively. Bars I to V A show that, if cleaning of the root canal is done only by means of a thorough wiping with five successive absorbent points, a continuous decrease is obtained in the number of cases which show growth inhibition. But even the fifth point shows growth inhibition in 49 cases (64%) after 24 hours incubation and in 29 cases (38%) after 72 hours incubation.

However, a cleaning by means of a Hedström file, followed by irrigation with 2 m.l. sterile saline solution and drying, produced a sharp decrease in the number of growth inhibition cases. The fourth point showed growth inhibition in 6 out of 52 cases (12%) after 24 hours incubation and in 2 cases (4%) after 72 hours incubation. (Bar IV B). The fifth point yielded growth in all 52 culture tubes (Bars V B). So did all controls (Bars VI).

In all cultures, which yielded growth, the same species of bacteria as used for inoculation, were recovered.

Discussion and conclusions

When using a polyantibiotic paste it appears that, if the cleaning of the root canal is limited to a thorough wiping, the absorbent point shows still growth inhibition when the canal is considered – in the clinical sense – ready for taking a culture. This is in accordance with the findings of BUCHBINDER and BARTELS (7), and those of BENDER and SELTZER (8).

The first point, used to wipe the root canal, contains so much of the antibiotic paste that almost 100% of the cultures show growth inhibition. The amount of paste decreases on each of the subsequent points as is demonstrated by the gradually decreasing percentage of growth inhibition cases. The fifth point, however, showed still growth inhibition in 38% of the cultures. The consistency of the results is an indication that the test-method has been satisfactory, i.e. the amount of inoculum has been sufficiently small to demonstrate different degrees of growth inhibition, and that the technique of cleaning is sufficiently reproducible.

This provided the possibility to find out whether the growth inhibition can be decreased significantly by means of additional cleaning methods. By using a Hedström file and irrigating with sterile saline the antibiotic paste could be removed effectively enough to produce cultures that showed no growth inhibition.

After application of desinfectants such as ChKM, chlorinated soda and hydrogen peroxide, these additional cleaning methods do not seem to be necessary. It is not advisable, however, in such cases to use the paper point, which has been used as a dressing in the root canal, for taking a culture, as advocated by MCPHEE (10).

What are the consequences for daily practice? We have to bear in mind that in the experiment, described in this paper, the amount of antibiotic paste carried by the paper point is large enough to inhibit the growth of bacteria, separately inoculated in the culture medium. At first, the concentration *in* the paper point certainly will be higher than in the culture medium although gradually the difference will disappear. The bacteria *in* the points are more likely to be subjected to the growth inhibiting effect than those, added to the culture medium. Obviously the first five points used for cleaning, are not suitable for taking cultures. A culture taken after irrigating the root canal shows no growth inhibition of separately inoculated bacteria. The question arises whether this is true also for bacteria, transported by the point from the root canal. The possibility exists that this point also contains traces of medicaments, too small to cause growth inhibition in the culture tube, but sufficient to inhibit the growth of a small amount of bacteria, present in the point.

In view of this, these tests do not provide absolute proof that no growth inhibition *can* occur.

The traces of medicament adhering to points after irrigating with saline will be small and will quickly be eluted and diluted in the culture tube. Furthermore the presence of very sensitive organisms in a canal that has been filled for a week with the concentrated medicament is hardly to be expected. For these reasons we are of the opinion that the technique described in this paper makes it possible to take cultures from treated canals without appreciable interference of growth inhibition.

The question arises, whether a culture taken from a root canal, out of which antibiotic paste has been eliminated in the described manner, is representative.

The bacteria, living in the canal and in the adjacent superficial layers of dentin will be destroyed by the paste or be washed out of the canal. However, what has happened to the bacteria in the deeper layers, the lateral branches and the peri-apical area?

Although this objection remains theoretically true, the following experiments seem to disprove its practical importance:

1. From a number of root canals, after taking a culture, a large mass of dentin was collected by means of a Gates glidden drill. After culturing these dentin particles no additional growth could be observed. (12)

2. Control cultures, taken after one week from once negative root canals, showed growth in a small percentage of the cases only (this percentage is the same in cases, where no antibiotics had been used previously) (11)

3. Furthermore, control cultures taken with adsorbent points, having been sealed in root canals during 1 week, 3, 6 and 12 months respectively (without a medicament) showed positive results in the same small percentage (12) as mentioned under 2.

These findings indicate that the afore mentioned objection cannot be of great importance.

Samenvatting

Het probleem van de groeiremming werd bestudeerd bij kweekproeven, genomen uit wortelkanalen behandeld met polyantibiotica, chloorphenol-kamfer-menthol (ChKM, Walkhoff) of Na-hypochloriet en waterstofperoxyde. Een zeer klein inoculum gevoelige staphylococcen werd toegevoegd aan de voedingsbodem, waarin de points werden bebroed.

Na mechanische preparatie en irrigatie met Na-hypochloriet en waterstofperoxyde treedt nimmer groeiremming op, zelfs niet wanneer de points verzadigd zijn met deze desinfectantia.

Het is beter om bij het gebruik van ChKM de – in het kanaal afgesloten – tampon niet te gebruiken voor bebroeding. Points die benut zijn om het kanaal te drogen vertoonden in geen enkel geval groeiremming.

Betreffende de applicatie van antibiotica bleek:

1e. Wanneer men zich bij de reiniging van het kanaal beperkt tot grondig uitvegen met absorbent points, treedt zelfs bij de 5e point nog groeiremming op in 38% van de gevallen na bebroeding gedurende 72 uur.

2e. Door extra reiniging met behulp van een Hedströmvijl en irrigatie met 2 m.l. fysiologische zoutoplossing kan groeiremming voorkomen worden.

Ofschoon de toegepaste methode van onderzoek geen absoluut bewijs levert, dat geen groeiremming *kan* optreden, menen wij dat betrouwbare kweekproeven genomen kunnen worden uit wortelkanalen, wanneer na het gebruik van antibiotica een extra reiniging wordt toegepast door middel van irrigatie.

Summary

The growth inhibition in cultures taken from root canals treated with polyantibiotics, camphorated chlorphenol (ChKM, Walkhoff) or chlorinated soda and hydrogen peroxide was studied. A very small inoculum of sensitive staphylococci was used to inoculate the culture medium in which the points were incubated.

After mechanical cleaning and irrigation with chlorinated soda and hydrogen peroxide, no growth inhibition occurs even when points are used which are saturated with these desinfectants.

In the case of camphorated chlorphenol (ChKM, Walkhoff) the point that served as a dressing should not be used for culturing. Subsequent points employed to dry the canal never showed growth inhibition however.

As far as polyantibiotics are concerned it appeared that:

1. if the cleaning is done only by wiping the root canal with absorbent points, even the fifth point showed growth inhibition in 38% of the cases, after culturing during 72 hours.

2. by cleaning with a Hedström file and irrigation with 2 m.l. sterile saline solution, growth inhibition can be eliminated.

Although the test method, described in this paper, cannot provide absolute proof that no growth inhibition can occur we think that reliable cultures can be taken from root canals with polyantibiotics after cleaning by irrigation.

Acknowledgement

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