From:
The Department of Oral Diagnosis
and The Department of Microbiology,
The Royal Dental College,
Aarhus, Denmark

FALSE NEGATIVE CULTURES FROM ROOT CANALS
INHIBITION OF BACTERIAL GROWTH BY SOME DRUGS USED IN ROOT CANAL THERAPY

by

LENA MIKKELSEN
ELSE THEILADE

INTRODUCTION

When drugs are used in root canal therapy and bacteriological cultures are used as a guide for the treatment, it is important to realize that the drugs may cause false negative cultures. If the paper point is contaminated during sampling with drugs deposited in the canal the result may be inhibition of growth in the culture.

Such false negative cultures could be avoided if the drug was active only for a limited time after it was deposited in the canal. In the literature only incomplete or conflicting information is found concerning the duration of the activity of various nonspecific chemical disinfectants in root canals. *Engström* (1958) found camphorated phenol to remain active in the root canal for 5 days. *Uchin* and *Parris* (1963) found camphorated parachlorophenol to have a growth inhibiting effect after 7 days in 53 per cent and after 14 days in 37 per cent of the cases examined. According to *Grossman* (1965) camphorated chlorphenol had lost 22 per cent of its activity in 14 days. Antibiotics seem to remain active in root canals for extensive periods of time. Thus *Søndergaard* (1956) using paper points moistened with a 10

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per cent solution of Nebacetin®[1]) (a polyantibiotic consisting of neomycin and bacitracin in equal parts) found that points deposited in root canals for 14 or 42 days produced similar zones of inhibition on plates seeded with a staphylococcus. *Möller* (1966) reported chlortetracycline, oxytetracycline and chloramphenicol to remain active in the root canal for at least one month. The drugs generally used in root canal therapy thus appear to be active for periods longer than the normal intervals between sittings. Furthermore, such a prolonged activity is probably necessary to get the full beneficial effect of the drug.

The growth inhibiting effect of the drugs used therefore must be removed to obtain a reliable culture from the canal. In some cases this can be achieved by adding specific neutralizing substances to the growth medium such as penicillinase against penicillin, para-aminobenzoic acid against sulfonamides, thiosulfate against iodine and chlorine preparations, cysteine against streptomycin, and ferric chloride against phenol. However, very little is known about the efficiency of most of these neutralizing substances and of the concentrations needed to inactivate the drugs completely. Furthermore, for most of the drugs used in endodontic therapy no specific neutralizing agents are available.

Möller (1966) obtained a good non-specific inactivating effect on many drugs (except broad-spectrum antibiotics) by rinsing the root canal with a sampling fluid containing peptones and gelatine and by using charcoal impregnated paper points for sampling.

Other investigators have relied on a simple dilution of the drugs. Thus some authors (*Yates* and *Morse*, 1938) have used the medicated point to inoculate the culture and have considered the dilution of the drug in the culture medium to be sufficient to prevent inhibition of growth. Others (*Grossman*, 1965; *Zeldow*, 1965) have removed more or less of the drug by drying the canal with 2–5 paper points before sampling. Such procedures, however, are insufficient to prevent false negative cultures. This was shown by *Buchbinder* and *Bartels* (1951), who found inhibition of growth by antibiotics after drying the canals with 4 points and after dilution in the culture medium. A few authors (*Strindberg*, 1952; *Möller*, 1958), have recommended rinsing the canal with sterile water or saline to achieve a sufficient dilution of the drugs.

No precise information thus seemed to be available in the literature concerning the precautions necessary to avoid false negative cultures due to the presence of drugs in the bacteriological samples. It was therefore decided

[1]) H. Lundbeck & Co. A/S, Copenhagen.

to investigate the duration of activity of an antibiotic (Nebacetin®) and a non-specific chemical disinfectant (camphorated paramonochlorphenol) in root canals. Furthermore, it was attempted to elucidate the efficiency of drug removal by rinsing the canal with saline before the bacteriological sampling.

MATERIAL AND METHODS

Clinical methods

The material was collected during root canal therapy performed by dental students mostly on teeth with previously untreated necrotic pulps and in a few cases during revision of inadequate root fillings.

Root canal treatment was carried out under rubber dam and with sterile instruments. During instrumentation the canal was kept moist with sterile saline. After mechanical cleansing as well as in all subsequent sittings the canal was rinsed 5 times with 1 ml sterile saline in a syringe and dried with paper points after each 1 ml. Bacteriological sampling was performed as described by *Möller* (1958). The canal was dried with several paper points after the rinsing until only 2—3 mm of the tip of the point became moist. This paper point was transferred to a culture tube with Brain-heart infusion broth (Difco).[1]) The canal was again filled with saline and dried, and the last paper point (moistened only for 2—3 mm in the apical end) was transferred to a culture tube with Fluid thioglycollate medium (Difco). At the end of each sitting a cotton pellet moistened with camphorated paramonochlorphenol was placed in the pulp chamber and the root canal was sealed with a double seal of guttapercha and Cavit (ESPE).[2]) Seven days elapsed between each sitting and two consecutive negative cultures were required before root filling was performed.

In case of growth in the culture obtained during the second appointment the canal was treated with Nebacetin in the third sitting in the following manner. After the bacteriological sampling the canal was filled with Nebacetin® (a solution containing 50 mg neomycin sulfate and 2500 units of bacitracin per ml) by means of a small syringe and a cotton pellet moistened with Nebacetin was placed in the pulp chamber. Growth of yeast has been shown to occur in many cases when Nebacetin was left in the root canal between appointments (*Theilade* and *Schiött*, 1964). Therefore the Nebacetin was removed after 5 minutes by drying with paper points followed

[1]) Difco laboratories, Detroit, Michigan, USA.

[2]) ESPE, GMBH — Seefeld/Oberbay, Germany.

by rinsing with saline (5 times 1 ml in a syringe) and again drying the canal with paper points after each 1 ml. After this treatment a cotton pellet with camphorated paramonochlorphenol was placed in the pulp chamber as usual.

Measurement of antibacterial effect of camphorated paramonochlorphenol and Nebacetin in root canals

The antibacterial activity of camphorated paramonochlorphenol after 7 days in the root canal was determined in the laboratory. In cases where only camphorated paramonochlorphenol had been used, the medicated cotton pellet was removed from the pulp chamber and transferred to the surface of a blood agar plate (Blood agar base (Difco) with 10 per cent calf's blood) inoculated with a strain of Sarcina sensitive to camphorated paramonochlorphenol. Following this procedure a little exudate was collected from the apical part of the root canal with a paper point (1. point) which similarly was placed on the inoculated blood agar plate. Finally after rinsing the canal 5 times with 1 ml of saline and drying with paper points, the point which was only moist with liquid from the canal on the apical 2—3 mm (2. point) was also placed on the inoculated blood agar plate.

When Nebacetin was used in root canal treatment the antibacterial effect of Nebacetin still present in the canal after application was examined. One paper point was selected when the canal was dried after each of the 5 times 1 ml of saline used for rinsing after the Nebacetin had been applied. These 5 points were all moistened on the apical 2—3 mm with liquid from the canal and they were placed on a blood agar plate inoculated with a Nebacetin sensitive strain of *Pneumococcus*.

When possible the antibacterial activity in the Nebacetin treated root canals was examined again in the next appointment 7 days later. After removal of the cotton pellet with camphorated paramonochlorphenol a paper point was introduced into the root canal down to the apex (1. point) and then placed on a blood agar plate inoculated with the *Pneumococcus*. After rinsing 5 times with 1 ml of saline and drying with paper points, the point which was moist on the apical 2—3 mm (2. point) was transferred to the inoculated blood agar plate.

All the blood agar plates were incubated aerobically for 24 hours and the zones of inhibition around the pellets and paper points were measured.

RESULTS

A total of 27 root canals in 22 teeth were used in the investigation. All of the 22 cotton pellets moistened with camphorated paramonochlorphenol

Table I.

Number of samples from root canals showing zones of inhibition of growth due to camphorated paramonochlorphenol 7 days after deposition in root canals.

	+	±	—
Zone of inhibition from cotton pellet	18	4	0
Zone of inhibition from 1. paper point	7	5	14
Zone of inhibition from 2. paper point	0	4	23

\+ indicates a definite zone of inhibition of growth on the inoculated blood agar plate
— indicates no zone of inhibition
± indicates no definite zone but a certain decrease in the growth immediately surrounding the pellet or paper point.

showed antibacterial activity 7 days after they were placed in the pulp chamber (Table I). Immediately after removal of these cotton pellets active camphorated paramonochlorphenol was found in the apical part of 12 out of the 26 root canals examined (Table I, 1. point). After rinsing 5 times with 1 ml saline and drying with paper points after each 1 ml only 4 out of the 27 root canals showed traces of antibacterial activity (two of these four were from the same tooth, Table I, 2. point).

Nebacetin was far more difficult to remove from the root canals (Table II). The zone of growth inhibition around the 5. point (after rinsing 5 times with 1 ml saline and drying after each 1 ml) in most cases was smaller than that around the 1. point (after rinsing once with 1 ml saline and drying). Even the 5. point, however, showed antibacterial activity in 26 cases of the 29 examined (Table II). This indicates that rinsing with sterile

Table II.

Number of samples from root canals showing zones of inhibition of growth due to Nebacetin during rinsing with saline immediately after treatment with Nebacetin.

	+	±	—
Zone of inhibition from 1. point	22	3	1
Zone of inhibition from 2. point	20	6	0
Zone of inhibition from 3. point	16	13	0
Zone of inhibition from 4. point	12	15	1
Zone of inhibition from 5. point	10	16	3

Symbols +, ± and — as in Table I.

Table III.

Number of samples from root canals showing zones of inhibition of growth 7 days after treatment with Nebacetin, rinsing with saline and deposition of camphorated paramonochlorphenol

	+	±	—
Zone of inhibition from 1. point	9	1	3
Zone of inhibition from 2. point	0	0	13

Symbols +, ± and — as in Table I

saline was not efficient in removing Nebacetin in the same sitting as it was applied to the root canal. There would thus be a considerable risk of false negative cultures involved in taking bacteriological samples at this stage.

Seven days after the root canals were treated with Nebacetin and camphorated paramonochlorphenol was placed in the pulp chambers, growth inhibiting activity was found in 10 root canals of the 13 examined immediately after removal of the medicated cotton pellet (Table III, 1. point). With the method used it was impossible to decide whether the inhibition was due to Nebacetin or to camphorated paramonochlorphenol. However, after rinsing 5 times with 1 ml of saline and drying the canal with paper points after each 1 ml, no antibacterial activity could be detected in any of the 13 root canals examined (Table III, 2. point). Taking bacteriological samples following this rinsing procedure at this stage of the treatment thus should give no risk of inhibition of growth due to Nebacetin (Table III) and only an insignificant chance of inhibition due to remnants of camphorated paramonochlorphenol (Table I).

DISCUSSION

It is impossible to determine how much drug should be present on the point used for bacteriological sampling to give inhibition of growth in the culture. The paper point contains an unknown and varying number of microorganisms of varying sensitivity. The concentration of drug throughout the culture medium will differ being highest around the point, where also the microorganisms are found. With these possible sources of error in mind it is necessary to require the sampling points to be free from drugs. It is too hazardous to rely on the medium to give sufficient dilution to neutralize the antibacterial effect.

The present work has shown that the presence of antibacterial activity can be detected even 7 days after the drugs were applied to the root canals (Tables I and III, 1. point). *Bender et al.* (1954) reported paper points from root canals treated with antibiotics to produce false negative cultures in 13 per cent of the cases if the cultures were incubated at least 7 days, and in 32 per cent if the time of incubation was only 48 hours. The percentage of false negatives probably was even higher because some of the cases were left out during the selection of the material.

Efficient removal of drugs prior to bacteriological sampling is rarely carried out during the generally accepted procedures. Therefore false negative cultures due to antibacterial drugs (especially antibiotics) represent a serious problem in the reliability of root canal cultures. Camphorated paramonochlorphenol can be removed by a thorough rinsing in the same sitting (Table I, 2. point). Antibiotics such as Nebacetin are more difficult to remove, probably because they are active even after strong dilution (Table II). The rinsing procedure developed during this investigation seems to help in obtaining a reliable sample from root canals to which drugs have been applied.

The Nebacetin treatment is quite time consuming if reliable cultures are to be obtained. Furthermore, it may lead to proliferation of yeasts and development of resistant strains of bacteria. Also neomycin allergy is noted with increasing frequency by dermatologists. (*Hjorth* and *Thomsen*, 1966).

SUMMARY

False negative cultures due to the presence of growth inhibiting drugs in samples from root canals represent a serious problem often neglected in bacteriological control in root canal therapy. Cotton pellets moistened with camphorated paramonochlorphenol were placed in the pulp chamber of 22 teeth during root canal treatment. When the pellets were removed 7 days later they all showed antibacterial activity (Table I). Immediately after removal of the pellets active camphorated paramonochlorphenol was found in the apical part of 12 out of 26 root canals tested (Table I, 1. point). After rinsing the root canals 5 times with 1 ml of saline and drying with paper points after each 1 ml only traces of the drug were found apically in 4 root canals of 27 examined (Table I, 2.point). Nebacetin® (a solution containing neomycin and bacitracin) was placed in the root canals for 5 minutes followed by rinsing and drying as above. Even after the last rinsing Nebacetin® was present in samples from 26 root canals of the 29 tested (Table II, 5. point). Seven days after the root canals had been treated with Nebace-

tin® and pellets moistened with camphorated paramonochlorphenol had been placed in the pulp chamber, growth inhibiting activity could be detected in 10 of 13 root canals immediately after removal of the pellet (Table III, 1. point). After rinsing 5 times with 1 ml of saline and drying with paper points after each 1 ml the drugs were removed from all 13 root canals. Thus camphorated paramonochlorphenol has to be removed from root canals by thorough rinsing and drying before a reliable bacteriological sample can be obtained. Nebacetin® is even more time consuming to remove.

RÉSUMÉ

CULTURES FAUSSEMENT NÉGATIVES PROVENANT DES CANAUX RADICULAIRES. INHIBITION DE LA CROISSANCE MICROBIENNE PAR CERTAINS MÉDICAMENTS EMPLOYÉS LORS DES TRAITEMENTS DES CANAUX

Lors du contrôle bactériologique employé au cours des traitements radiculaires, les cultures faussement négatives dues à la présence dans les prélèvements de médicaments inhibiteurs de la croissance bactérienne représentent un problème sérieux mais souvent négligé. Dans 22 dents, des boulettes de coton imbibées de paramonochlorophénol camphré ont été placées dans la chambre pulpaire pendant le traitement radiculaire. Lorsque les boulettes de coton on été enlevées, 7 jours plus tard, elles présentaient toutes une activité antibactérienne (tableau I). Immédiatement après l'enlèvement des boulettes de coton, il était possible de mettre en évidence la présence de paramonochlorophénol camphré actif à la partie apicale de 12 des 26 canaux radiculaires testés (tableau I, 1.). Après avoir rincé les canaux à 5 reprises avec 1 ml de solution saline, et après les avoir asséchés avec des pointes de papier après chacun des rinçages d'1 ml, il a seulement été possible de trouver des traces du médicament au niveau de l'apex dans 4 des 27 canaux examinés (tableau I, 2.). Pendant 5 minutes, de la Nébacétine® (solution contenant néomycine et bacitracine) à été placée dans les canaux radiculaires, puis ceux-ci ont été rincés et asséchés comme ci-dessus. Dans 26 des 29 prélèvements faits dans les canaux radiculaires, on trouvait encore de la Nébacétine® après le dernier rinçage (tableau II, 5.). Sept jours après que les canaux aient été traités à la Nébacétine® et que des boulettes de coton imbibées de paramonochlorphénol camphré aient été placées dans la chambre pulpaire, il était possible de mettre en évidence une activité inhibitrice de la croissance microbienne immédiatement après l'enlèvement de la boulette de coton dans 10 des 13 canaux radiculaires. Après rinçage à 5 reprises avec 1 ml de solution saline et assèchement avec des pointes de papier après chacun des rinçages d'1 ml, les médicaments

avaient été éliminés des 13 canaux. Ainsi, il est nécessaire d'éliminer le paramonochlorphénol camphré des canaux par rinçage et assèchement minutieux avant qu'il soit possible d'obtenir un prélèvement bactériologique auquel on puisse se fier. La Nébacétine® prenait encore plus de temps à éliminer.

ZUSAMMENFASSUNG

FALSCHE NEGATIVE KULTUREN IN WURZELKANALPROBEN. BAKTERIENWACHSTUMSHEMMUNG EINIGER WURZELKANALMEDIKAMENTE

Falsche, negative Kulturen wegen Wachstumhemstoffe in Wurzelkanalproben repräsentieren ein ernstes Problem, das sehr oft in der bakteriologischen Kontrolle der Wurzelkanalbehandlung negligiert wird. Wattepellets mit kamferiertem Paramonochlorphenol durchnässt wurden während der Wurzelkanalbehandlung an den Kanaleingang von 22 Zähnen eingelegt. Sieben Tage später herausgenommen zeigten alle Wattepellets antibakterielle Tätigkeit (Tab. I). Gleich nachher wurden aktives kamferiertes Paramonochlorphenol in Wurzelspitzen von 12 aus 26 geprüften Wurzelkanalen gefunden (Tab. I, 1. Punkt). Nach fünf Wurzelkanalspülungen mit 1 ml physiologischer Kochsalzlösung und Trockenlegung mit Papierspitzen nach jeder Spülung, wurden nur Spuren vom Medikament in 4 aus 27 untersuchten Wurzelkanalspitzen gefunden (Tab. I, 2. punkt). Nebacetin® (eine Lösung von Neomycin und Bacitracin) wurde 5 Minuten in den Wurzelkanal eingelegt; danach wurde Spülung und Trockenlegung wie oben erwähnt durchgeführt. Nach der letzten Spülung und Trockenlegung war Nebacetin® in den Proben von 26 aus 29 Wurzelkanalen gefunden. Sieben Tage nach der Nebacetinbehandlung des Wurzelkanals und nach dem Einlegen von Wattepellets mit kamferiertem Paramonochlorphenol an den Kanaleingang, war in 10 aus 13 Wurzelkanalen wachstumstörende Aktivität sofort nach der Entfernung der Wattepellets zu erkennen (Tab. III, 1. Punkt). Nach fünf Spülungen mit physiologischer Kochsalzlösung und Trockenlegung mit Papierspitzen nach jeder Spülung, waren die Medikamente aus allen 13 Wurzelkanalen entfernt. Deshalb muss kamferiertes Paramonochlorphenol durch sorgfältige Spülung und Trockenlegung aus dem Wurzelkanal entfernt werden bevor eine zuverlässige bakteriologische Probe ausgenommen werden kann. Das Entfernen von Nebacetin® erfordert sogar mehr Arbeit.

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Address:

Royal Dental College,
DK-8000 Aarhus C, Denmark