

HISTO-PATHOLOGICAL AND HISTO-BACTERIOLOGICAL STUDIES OF
THE RELATION BETWEEN THE CONDITION OF STERILIZATION
OF THE INTERIOR OF THE ROOT CANAL AND THE HEALING
PROCESS OF PERIAPICAL TISSUES IN EXPERIMENTALLY
INFECTED ROOT CANAL TREATMENT*

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Abstract

Four kinds of up-to-date typical disinfectants and calcium hydroxide paste were used in the sterilization and filling of a total of 215 infected root canals which had been experimentally created in the teeth of 17 dogs; and the relation of the effect of sterilization in the root canal and the healing condition in the periapical tissues was histopathologically and histobacteriologically studied. It was clarified that these disinfectants are effectual only on the surface layer of the wall of the root canal, being almost impossible to exterminate bacteria in the deep part of the wall and the branch of the root canal or in the cementum lacunae. This bacteria resumes a propagation towards the surface layer as the effect of sterilization weakens. Calcium hydroxide paste was proven to be an excellent filling material, having the effect of accelerating the natural healing functions in the periapical tissues. Bacteria living in the depth of the tissues was clearly observed to diminish and finally to disappear as the healing progressed. Also, a sufficient enlargement of the root canal is remarkably efficacious in the extermination of bacteria. Therefore, an overestimation of chemical sterilization in the case of root canal sterilization should be carefully evaluated.

INTRODUCTION

A great number of clinical and basic studies have been made concerning the treatment of the infected root canal, one of the great problems in dental practice, but no satisfactory result has been obtained.^{1-7, 9)}

It may be that this failure is due chiefly to the fact that, up to the present, studies have been limited to the investigation of certain small spheres and have therefore missed any broad synthetic study, especially in that the root canal sterilization and filling materials, which are the central features of this treatment, have never been considered together.

In other words, the researchers who hold the clinical and bacteriological viewpoint maintain that the main feature of this treatment is manual complete sterilization of the root canals^{6,9)} and they regard filling as a mere mechanical closure for the pre-

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vention of reinfection. On the other hand researchers who follow the lines of pathology, being doubtful of the possibility of complete sterilization of the root canals which are anatomically very complicated, insist that the primary and essential point is the employment of such canal filling materials as will quicken the natural healing function of the apical periodontal tissues in order to perform the indirect sterilization which is necessary for complete healing.^{8, 10-12)}

Although it is plain that the solution of this problem can be found only through a synthetic observation made from various viewpoints, no good work of such detailed investigation has been published.

This being the case, the writer tried to throw light on this problem by experimenting with infected root canals in dogs' teeth, applying various treatments, and then patho-histologically and histo-bacteriologically inspecting the relation between the conditions of sterilization of the root canals and the states of the healing process of the periapical tissues during the experiment.

MATERIAL AND METHODS

A total of 215 infected root canals were obtained by exposing with dental instruments the root canals of the teeth of 17 dogs, and leaving them for 20-83 days in a situation in which they would be naturally infected. Moreover, it was roentgenologically proven that almost all of these infected cases had an inflammatory focus at their apical region.

Among these artificially infected root canals, 172 cases were applied with Phenol-Camphor, Eugenol, 7000 u. Procain-Penicillin G. in oil, or 25 mg/cc Terramycin, while 21 cases were applied only with sterile physiologic saline. Furthermore, no treatment was given to the other 22 cases.

Then, the root canals were filled with calcium hydroxide paste, which had been made by sterilizing calcium hydroxide by high pressure and softening it with sterilized water. Finally, the top of the root canal was closed with amalgam.

The conditions immediately after, 25 days after and 50 days after the root canal filling were observed in 58 cases, 70 cases and 44 cases respectively.

All the above-mentioned materials were fixed in a formalin solution demineralized in alcohol nitrate, prepared into celloidin-embedded sections by the common method and, after staining either by hematoxylineosin, Gram-Weigert's method or Goodpasture-MacCallum's method, were observed with a microscope.

RESULTS

First, neither histopathologically nor histobacteriologically was any fundamental

difference observed in the conditions of infected root canal and chronic apicalis periodontitis in human teeth from those in the 22 experimental infected root canals and the subsequent chronic apicalis periodontitis (9 cases of alveolar abscess and 13 cases of granuloma) in dogs. (Fig. 1)

A) Dentine. B) Cementum. C) Purulent foci opposite the apical ramifications. D) Inner layer of abscess wall (pyogenic membrane). E) Mass of pus cells. F) Infectious dirt. G) Outer layer of abscess wall (fibrous membrane). H) Contents of abscess cavity (necrotic tissue, bacterial mass, etc.). I) Alveolar bone with lacunar absorption.

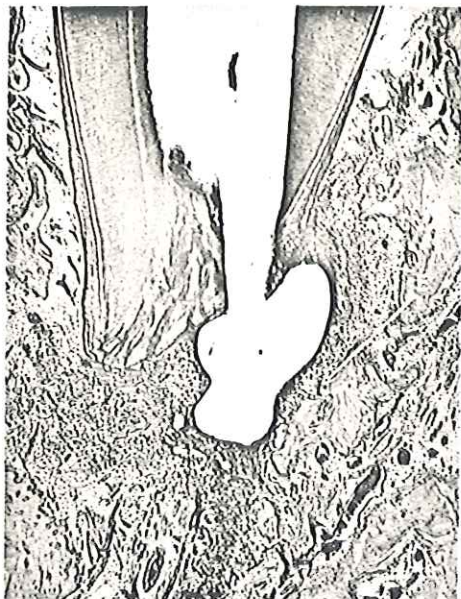


Fig. 1. Experimentally infected root canal of a dog and its subsequent disease, periodontitis apicalis purulenta chronica (chronic alveolar abscess): Condition after root canal has been left open for 83 days. Hematoxyline-eosin staining, x20.

Next, histopathological and histobacteriological findings in 172 cases on which the above-mentioned treatment had been given and which were observed after various lengths of time were as follows:

In 58 cases observed immediately after the root canal filling, almost without exception, chronic apicalis periodontitis remained, frequently accompanying traumatic changes in the alveolar membrane and in the alveolar bone due to mechanical stimulation, unintentionally given during the treatment. Moreover, nearly all cases showed bacterial infection in many places in the cementum lacunae, branches of the root canal and the walls of the root canal. Concerning these findings there were no special differences among the result of 4 kinds of disinfectants. (Figs. 2, 3)

In the observation of 70 cases 25 days after the root canal filling, the number of cases showing tendencies towards healing of the inflammatory focus, such as proliferation and fibrotic change in the granulation tissues, regeneration of the alveolar bone,



- A) Apical ramification filled with bacteria. B) Small abscess formed opposite the the apical ramification. C) Deeply spread pyogenic membrane. D) Broken fibre bundle. E) Partly absorbed alveolar bone.

Fig. 2. Condition immediately after root canal filling: Case treated with eugenol. (An example of bad healing.) Hematoxyline-cosin staining, x20.



Fig. 3. Part 'A' of Fig. 2 under higher magnification. Gram-Weigert's staining, x640.

A) Apical ramification. B) Necrotic outer layer of root canal polyp. C) Pyogenic process spreading to the outer surface of the alveolar bone. D) Abscess cavity.

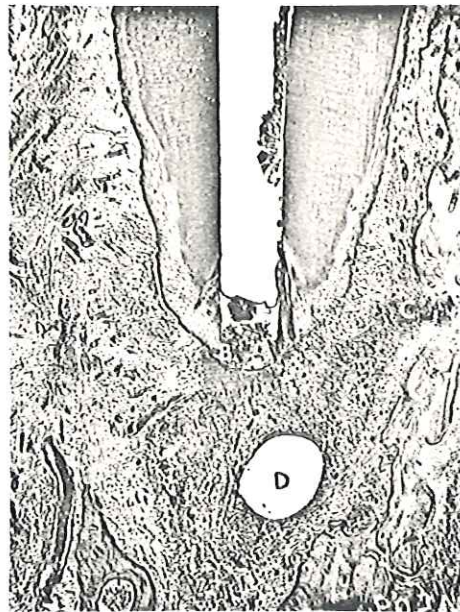


Fig. 4. Condition immediately after root canal filling: Case treated with terramycin. (An example of bad healing.) Hematoxylin-eosin staining. x13.

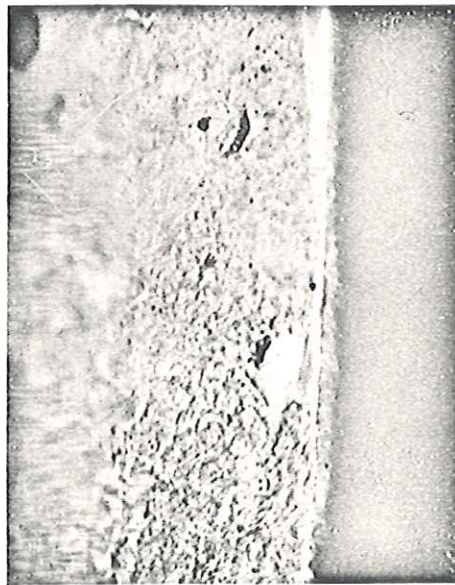


Fig. 5. Contents of root canal of Fig. 4. under higher magnification. Gram-Weigert's staining. x640.



A) Apical ramification. B) Inflammatory focus opposite the apical ramification. C) Root canal polyp. D) Periapical tissues almost without inflammatory cells.

Fig. 6. Condition 25 days after root canal filling: Case treated with phenol-camphor. (An example of good healing.) Hematoxyline-eosin staining, x19.



A) Infectious dirt in the root canal.
B) Growing purulent foci.

Fig. 7. Condition 25 days after root canal filling: Case treated with phenol-camphor. (An example of bad healing.) Hematoxyline-eosin staining, x14.

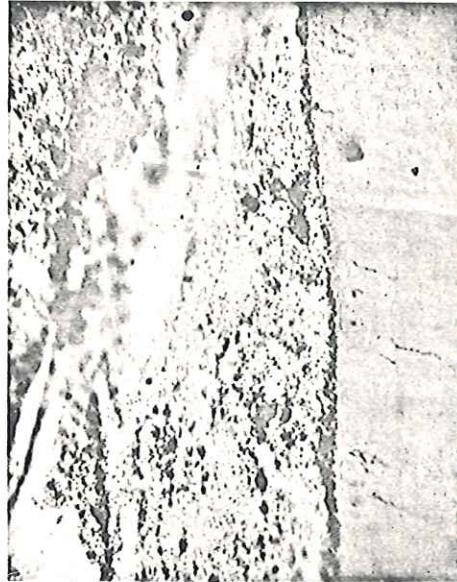
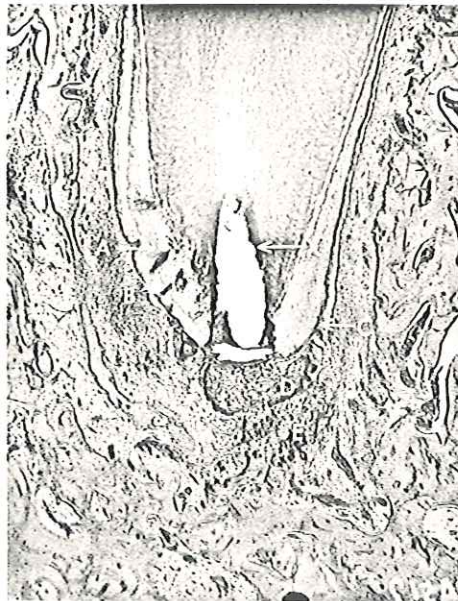


Fig. 8. Part 'A' of Fig. 7. under higher magnification, showing bacteria in wall and contents of root canal. Gram-Weigert's staining, x640.



A) Apical ramification containing origin of infection. B) Inflammatory focus due to the apical ramification. C) Proliferated cementum. D) Calculous deposit. E) Irregular calculous deposit.

Fig. 9. Condition 25 days after root canal filling: Case treated with eugenol. (An example of good healing.) Hematoxyline-eosin staining, x18.



Fig. 10. Part 'X' of Fig. 9. under higher magnification: Gram-Weigert's staining, x640.



A) Apical ramification still containing bacteria. B) Apical focus becomes almost completely fibrous. C) Calcium globers' deposit. D) New bones.

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Fig. 11. Condition 25 days after root canal filling: Case treated with terramycin. (An example of good healing.) Hematoxyline-eosin staining, x14.

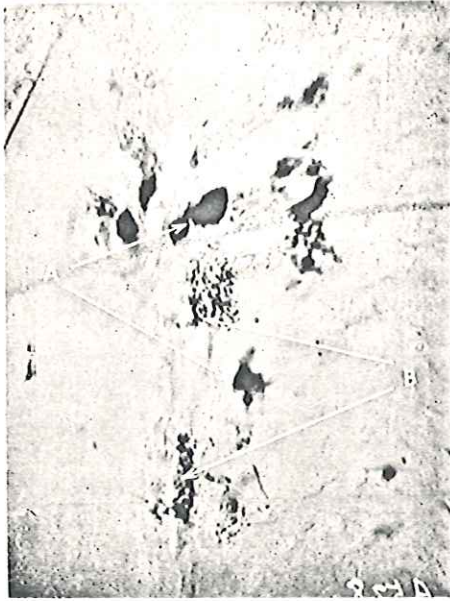


Fig. 12. Part 'A' of Fig. 11. under higher magnification. Gram-Weigert's staining, x640.

A) Dentine bridge during formation.
B) So-called cicatrized root canal polyp.
C) New hard tissue. D)
New bone.

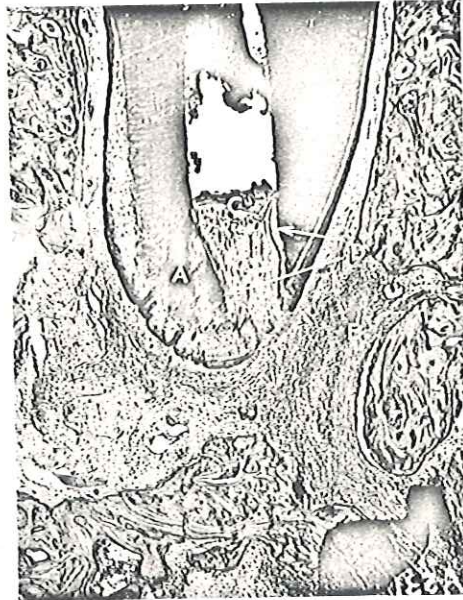


Fig. 13. Condition 25 days after root canal filling: Case treated with physiological salt solution. (An example of good healing.) Hematoxyline-eosin staining, x15.



A) Cementum lacunae. B) Ramifications.

Fig. 14. Bacteria in apical ramifications and cementum lacunae. Gram-Weigert's staining, x630.



A) Apical ramification. B) Purulent focus opposite the ramification. C) So-called dentine bridge. D) Reinforced new cementoid tissue. E) Remains of the apical foci.

Fig. 15. Condition 50 days after root canal filling: Case treated with phenolcamphor. (An example of bad healing.) Hematoxyline-eosin staining, x14.

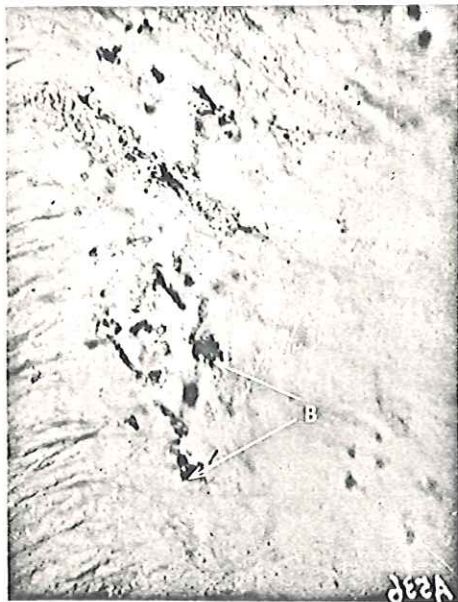


Fig. 16. Masses of bacteria remaining in ramification: Part 'A' of Fig. 15. Gram-Weigert's staining, x640.

A) Apical ramifications. B) Inflammatory foci opposite the ramifications. C) Root canal polyp. D) Hard tissue almost closing the apex.



Fig. 17. Condition 50 days after root canal filling: Case treated with eugenol. (An example of good healing.) Hematoxyline-eosin staining, x19.



A) Apical ramification. B) Cementum lacunae.

Fig. 18. Part 'A' of Fig. 17. Masses of bacteria attaching in apical ramification and cementum lacunae. Gram-Weigert's staining, x640.

A) Deeply spreading abscess. B) Root canal polyp rich in cells. C) Ulcerative surface of the polyp.

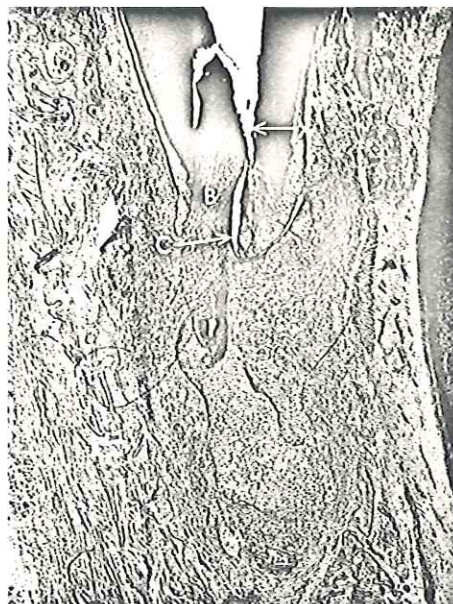
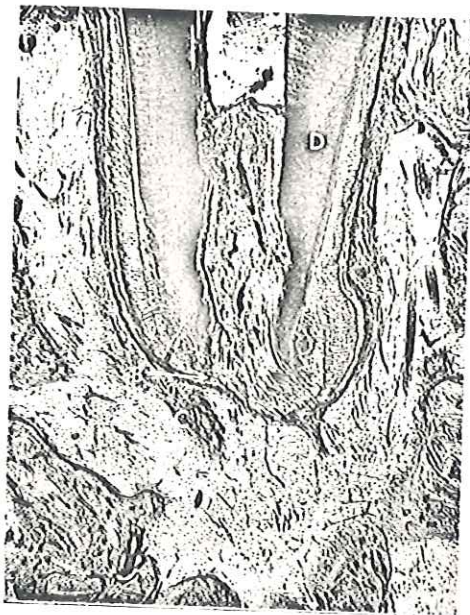


Fig. 19. Condition 50 days after root canal filling: Case treated with eugenol. (An example of bad healing.) Hematoxyline-eosin staining, x19.

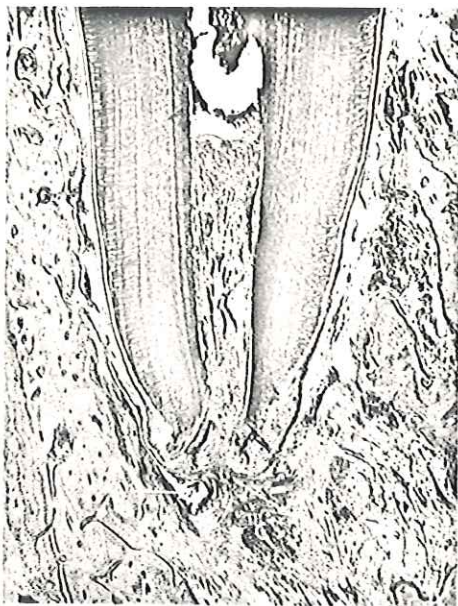


Fig. 20. Part 'X' of Fig. 19 under higher magnification. Gram-Weigert's staining, x640.



A) So-called cicatrized root canal polyp. B) Apical ramification closed by hard tissue. C) Slight remaining cell infiltration. D) Dentine bridge. E) New cementoid tissue. F) Almost normal apical periodontal membrane.

Fig. 21. Condition 50 days after root canal filling: Case treated with procain-penicillin G in oil. (An example of good healing.) Hematoxyline-eosin staining, x14.



A) Bleeding focus. B) Cicatrized root canal polyp. C) Remains of cell infiltration.

Fig. 22. Condition 50 days after root canal filling: Case treated with terramycin. (An example of good healing.) Hematoxyline-eosin staining, x10.

A) Root canal polyp. B) Apical ramification containing origin of infection. C) Inflammatory focus opposite the ramification. D) Pus cell layer exuded into the root canal. E) Purulent focus.

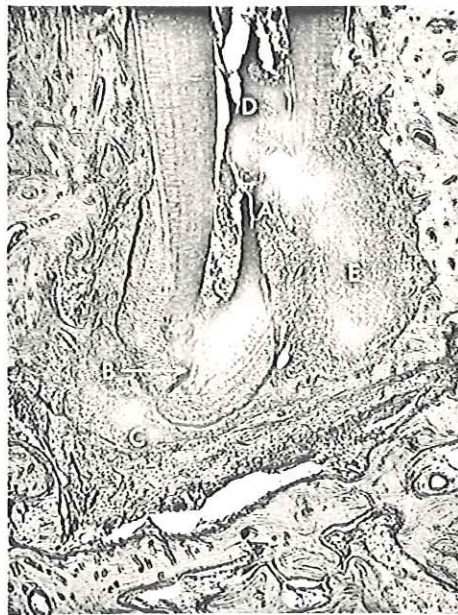


Fig. 23. Condition 50 days after root canal filling: Case treated with terramycin. (An example of bad healing.) Hematoxyline-eosin staining, x18.

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etc, were observed to have greatly increased. Also bacteria in the root canal was in general less in this group than in the group observed immediately after the filling (Figs. 9, 10, 11, 12). In some of the cases sterilized with phenol-camphor or 7,000 u. procain-penicillin G, however, bacterial infection was on the contrary greater than in the cases observed immediately after the filling (Figs. 7, 8). In general 25 mg/cc terramycin propylen-glycol and eugenol brought about a remarkable reduction of bacteria in the root canal; then phenol-camphor and lastly 7,000 u. procain-penicillin G. They were not, however, of great consequence, for there was absolutely no finding which could prove a specially close parallel relationship between the degree of bacterial infection in the root canal and healing in the periapical inflammatory focus, in all the cases in which various kinds of disinfectants were experimented upon.

In 44 cases observed 50 days after the root canal filling, further progress of healing in the periapical inflammatory focus, and obvious reduction of bacteria in the root canal were observed in general. The order of the effects of the disinfectants was the same as in the above mentioned cases. Frequently, however, bacteria was observed to have remained in many places such as cementum lacunnae, branches of the root canal and walls of the root canal even after healing in the periapical inflammatory focus had shown great progress. (Figs. 15, 16, 17, 18.) On the other hand, there were not a few cases in which the periapical inflammatory focus had healed complete-



Fig. 24. Part 'D' of Fig. 23. under higher magnification: Mass of bacteria increasing in the necrotic tissue. Gram-Weigert's staining, x640.

ly with total extinction of bacteria both from the root canal and the apical region. (Figs 21, 22.) These findings are considered to prove that the bacteria in deep parts of the root canal is destined to diminish as healing of the inflammation gradually takes place. In other words, healing of the periapical inflammatory focus progresses without much difference whether bacteria exists or not in the walls of the root canal, and bacteria diminishes subsequent to the healing of the tissues, generally beginning from the surface layer and progressing to the deep part of the root canal.

Histopathological and histobacteriological findings in the 21 cases in which, instead of the root canal disinfectants, physiologic saline was used in sterilization, show that the process of healing in the periapical inflammatory focus and diminution of bacteria in the root canal were not basically different from those in the cases in which the above-mentioned disinfectants were applied (Figs. 13, 14.).

Furthermore, among the cases sterilized with phenol-camphor, those which were inspected 25 days after root canal filling, except for 13 cases, had all been filled without exerting sufficient enlargement and sterilization of the root canal. In comparing condition of bacterial infection in the root canal in these cases to that of the 13 cases whose root canals had been sufficiently enlarged, the condition of the former was generally more severe than that of the latter. The severeness was especially remarkable in the main root canal and cementum lacunnae. Healing in the periapical inflammatory focus was also obviously slower in the former (Figs. 7, 8.).

DISCUSSION

In performing the infected root canal treatment, the traditional concept upon which all former studies have been made, is that an absolutely necessary pre-condition for the fast recovery from the periapical inflammatory focus is an artificial and perfect sterilization of the root canal. As the result of this study, however, it was proven that after the chemical sterilization by the technique generally used in the present day, although the surface layer of the walls of the root canal is thoroughly sterilized by it, bacteria is frequently alive in the deep areas, such as branches of the root canal, cementum lacunnae and the interior of the dentinal tubule and resumes propagation. There is no doubt, therefore, that the results from the bacteriological examination immediately after the root canal treatment, which have frequently been made public, are mere records of the temporary and superficial conditions and not evidence of the temporary and superficial conditions and not evidence of the entire work of sterilization in the root canal.

Next, the time relationship between diminution of the infectious bacteria remaining in the root canal after the root canal filling and healing process of the periapical

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inflammatory focus, was for the first time truly explained by this study, that is, that apical inflammatory disease heal prior to the complete extinction of the bacteria in the above-mentioned deep parts of the root canal tissues. Bacteria disappears naturally as inflammation heals. In other words, that which chiefly works in the healing of the infected root canal is the natural healing function of the periapical tissues. Also it was actually proven that, as healing progresses, the favorite culture media of bacteria, such as inflammatory exudate, destroyed tissue particles, etc. gradually decrease, and together with the active movement of histiocyte and leucocyte, bring about the complete extinction of bacteria.

The above-mentioned order of function can be called an indirect bactericidia compared with the direct bactericidia, which is the application of chemicals. Furthermore, due to the complicated shape and the microscopical structure of the root canal, it is almost impossible for any direct bactericidia to reach the bottom of it. It should be indirect bactericidia that occupies the most important position in sterilization of the infected root canal. Therefore, from this point of view, it is easy to understand that in order to achieve the purpose of infected root canal treatment, the most important thing is to choose such filling material as has a strong effect of accelerating the natural healing function of the periapical tissues.

On the other hand, the significance of the root canal sterilization by the application of chemicals is considered to be just a supplementary method for letting the biological effect of the filling material do its utmost, so as to make the periapical tissues heal completely.

Lastly, the significances of the mechanical enlargement and the physical and chemical cleansing to the root canal are: a) to remove the extremely dirty dentine and filthy contents of the root canal which are impossible to eradicate by chemical sterilization alone, b) to provide enough space for exudate and gas to issue from the periapical inflammatory focus and c) to make it easy for the filling material to give full scope to its biological effect on the periapical tissues. Therefore, whether the enlargement and cleansing were thoroughly performed or not is just as important as the choice of filling material in performing a successful treatment of infected root canal.

CONCLUSIONS

- 1) By the methods of sterilization of the root canal which are now generally employed, traces of bacteria can always be found not only in the apical ramifications, the cementum lacunae and the dentinal tubules, but they were also frequently observed in the principal root canals immediately after sterilization.
- 2) The sterilizing influence of antiseptics in the root canal is mostly superficial and

temporary. This tendency is specially strong in penicillin, which has recently been greatly favoured by some clinicians. Those which show a comparatively good effect or long-sterilizing influence are eugenol and terramycin; phenol-campher comes next.

- 3) As the effect of the antiseptics decreases, bacteria that have remained in the deep portions begin to proliferate and spread, especially in the principal root canals. It is conceivable that this is the beginning of the so-called reinfection of the root canal about which there has been so much discussion.
- 4) The result of the test of a culture of bacteria performed immediately after the completion of sterilization of the root canal does not represent the real state of infection of the interior of the root canal.
- 5) It is impossible for any known drug to achieve complete sterilization of the interior of the root canal, when it is used exclusively.
- 6) The healing of the periapical tissues after a treatment by the application of antiseptics alone is only poor, but also frequently causes inflammation due to chemical stimulus.
- 7) Bacteria remaining in the root canal gradually diminish as the inflammation of the periapical tissues heals. Therefore, sterilization of the root canal should be done not only by the direct method by drugs, but also indirectly by the discontinuation of the nourishment supply for the bacteria through the healing of the inflammation and the natural eradication of the remaining bacteria through the influence of histiocytes, leucocytes, etc. Especially in root canal treatment the latter ought to be considered as having more effect than the former.
- 8) The filling material is extremely important in treating an infected root canal. Therefore, in order to perform a successful treatment, a good filling material which has the power of promoting the natural healing function of the periapical tissues is essential.
- 9) Sufficient enlargement and cleansing of the root canal are very necessary for subduing bacterial infection of the interior and the wall of the root canal. Moreover, they help a great deal in allowing the canal filling materials to take effect.
- 10) Sterilization of the root canal in the treatment of the infected root canal should be considered as a supplementary and preparatory means of promoting the natural healing function of the periapical tissues so that they may ultimately be healed completely.

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