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# STUDIES ON ROOT CANAL MEDICAMENTS

## I. CYTOTOXIC EFFECT OF ROOT CANAL ANTISEPTICS

*by*

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## INTRODUCTION

The medicamentous treatment of the root canal covers the use of various antiseptics during the biomechanical instrumentation and irrigation of root canals and of dressings between sessions. In addition to an efficient antibacterial action these agents shall not irritate the vital tissue with which they come into contact. Various methods of examining the toxicity of antiseptics have been reported (*Reddish,* 1957), but it would seem that no studies have been published in which the cytotoxic effect of root canal antiseptics has been examined on HeLa cells. These cells have been used by *Rappaport et al.* (1964) in a study of the toxicity of endodontic filling materials.

## MATERIAL AND METHOD

The medicaments examined were the following:

Iodine-potassium iodide, 2 per cent (iodine 2, potassium iodide 4, distilled water 94)

Iodine-potassium iodide, 5 per cent (iodine 5, potassium iodide 10, distilled water 85)

Biosept®, 0.1 per cent (quartenary ammonium compound: cetylpyridine chloride; aqueous solution)

Biosept®, 1.0 per cent (see above)

Iodopax®, 0.04 per cent (iodofor: iodine dissolved in octylphenoxypolyglycol ether; aqueous solution)

Grossman's solution (zinc iodide 32, iodine 2.6, distilled water 65.4)

Chloramine, 5 per cent (chloramine T, aqueous solution)

Dakin's solution (2 per cent sodium bicarbonate solution mixed with calcium hypochlorite solution; freshly made)

Chlumsky's solution (phenol 30, camphor 60, 96 % alcohol 10)
Tricresol formalin (tricresol 10, formaldehyde solution 90)
Eugenol.

Since Chlumsky's solution, tricresol formalin, Dakin's solution and eugenol are not soluble in water, all the solutions were prepared in Parker 199 (State Bacteriological Laboratory, Stockholm) with an emulsifying agent namely Tween 80®, 0.1 per cent strength (sorbimacrogololeate 300). When the solution had been prepared calf serum was added until it contained 25 per cent. The concentration of the medicament that is reported in the results and tables is that before the serum was added.

The HeLa cells were suspended in Parker 199 containing 2 per cent of calf serum to give a cell concentration of 150,000 per millilitre.

The cytotoxic effect of the medicament was tested afterwards on HeLa cells by culturing on slides in culture chambers by the procedure described by *Bergman* (1959, 1963). In this method a glass ring is affixed to the slide with melted vaseline to provide a culture chamber with a volume of about 0.8 ml (Fig. 1). The method has been used by *Spångberg* (1966) in a study of root filling materials.

In the culture chambers 0.2 ml of the medicament solution was mixed with 0.5 ml of the cell suspension, and the medicament

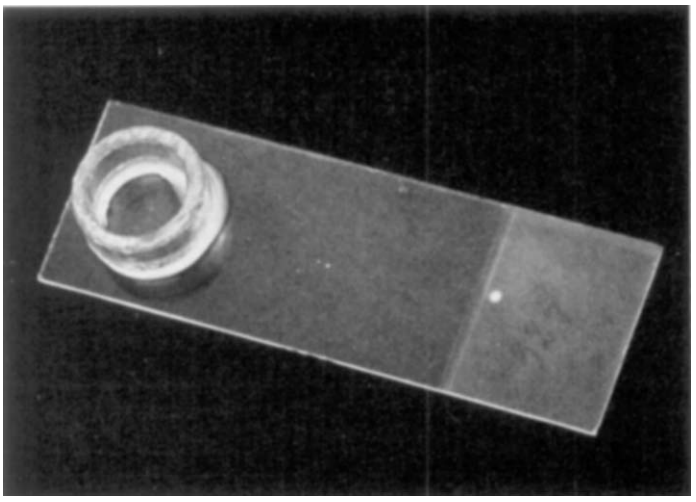

Fig. 1. Slide with culture chamber (Bergman's method).

was allowed to act on the cells in the solution for 6 hours at 37° C. In this period viable cells adhere to the slide.

The glass rings were then removed and the test solution washed with Parker 199 at 37°. The mats of cell on the slide were cultured in Hellenthal cuvettes for 24 hours at 37° in an 8 per cent carbon dioxide atmosphere. The culture medium was Parker 199, to which 5 per cent of human and 5 per cent of calf serum had been added.

The culturing was usually performed with 5 strengths of the medicament in ascending and descending series from 0.1 per cent (0.1, 0.2, 0.4 . . . and 0.1, 0.05, 0.025 . . .). On each occasion the culturing was performed on 2 slides for each concentration. The tests were repeated 5 times; so that each concentration was tested on 10 slides.

For comparison and to check the condition of the HeLa cells at each experiment a test with phenol in the concentrations 0.1, 0.2, 0.4, 0.8 per cent was performed. In addition, checks were made on calf serum and Parker 199 with 0.1 per cent Tween 80®.

At the end of the culture period the specimens were fixed and stained, and the examinations and cell counts were then performed (*Bergman,* 1963). The count was made under a phase contrast microscope.

## RESULTS

The total number of mitoses on 20 fields of vision and the mean value of cells on 20 fields of vision per slide were determined (Table I, Figs. 2 and 3).

The control glasses with calf serum and 0.1 per cent Tween 80® showed dense growth of cells.

It is seen from Table I and figures 2 and 3 that, in a concentration of 0.8 per cent, phenol exerted a cytotoxic effect on HeLa cells. The same applies to 2 per cent iodine-potassium iodide in a concentration of 12.8 per cent, to 5 per cent iodine-potassium iodide, conc. 3.2 per cent, to 0.1 per cent Biosept®, conc. 3.2 per cent, to 1 per cent Biosept®, conc. 0.4 per cent, to 0.04 per cent Iodopax®, conc. 12.8 per cent, Dakin's solution, conc. 12.8 per cent, 5 per cent chloramine, conc. 6.4 per cent, Chlumsky's solution, conc. 1.6 per cent. Grossman's solution,

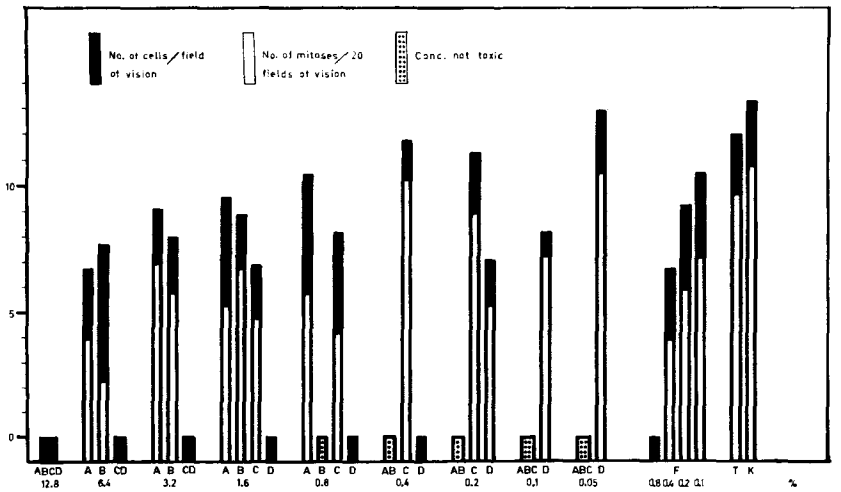


Fig. 2. Effect of root canal medicaments on HeLa cells.

A, 0.04 per cent Iodopax®
B, Dakin's solution
C, 0.1 per cent Biosept®
D, 1.0 per cent Biosept®

*Controls*

F, phenol
T, Tween 80®
K, calf serum

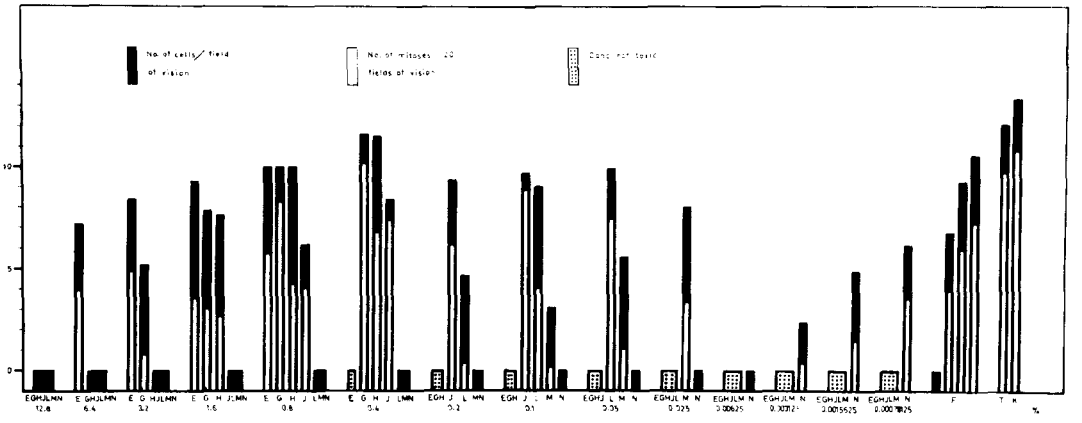

Fig. 3. Effect of root canal medicaments on HeLa cells.

E, 2 per cent iodine—potassium iodide
G, 5 per cent chloramine
H, 5 per cent iodine—potassium iodide
I, Chlumsky's solution
L, eugenol
M, Grossman'ssolution
N, tricresol formalin

*Controls*

F, phenol
T, Tween 80®
K, calf serum

**Table II**

*Ratio between toxic cencentrations of medicament and phenol*

| | |
|---|---|
| Tricresol formalin | 1/128 |
| Grossman's solution | 1/4 |
| eugenol | 1/2 |
| 1 per cent Biosept® | 1/2 |
| Chlumsky's solution | 2/1 |
| 0.1 per cent Biosept® | 4/1 |
| 5 per cent Iodine—potassium iodide | 4/1 |
| 5 per cent chloramine | 8/1 |
| Dakin's solution | 16/1 |
| 2 per cent iodine—potassium iodide | 16/1 |
| 0.04 per cent Iodopax® | 16/1 |

conc. 0.2 per cent, tricresol formalin, conc. 0.00625 per cent, and to eugenol, conc. 0.04 per cent.

Compared with phenol the cytotoxic effect was greatest for tricresol formalin. Grossman's solution, eugenol and 1 per cent Biosept® also had a stronger effect than phenol, while the other medicaments showed a lower toxicity (Table II).

## DISCUSSION

The cytotoxic effect of the medicaments was examined by culturing on microscope slides in a culture chamber by *Bergman*'s method (1959, 1963). Since several of the medicaments were not soluble in water an emulsifying agent (Tween 80®) was used throughout. It was found at the control tests performed for each experimental series that at the concentration used this agent did not affect the HeLa cells appreciably (Figs. 2 and 3).

As the cytotoxic effect on HeLa cells, which are epithelial cells isolated from cancer tissue, would seem to be similar to that for normal cells (*Bergman,* 1966) it would seem justified to draw general conclusions from the results.

To obtain even cell mats on the slide it was found advantageous to add calf serum. Without the addition of serum and in the presence of antiseptics and Tween 80® the cells adhered at the periphery of the culture chambers and grew sparsely and unevenly at the centre.

The concentrations of the stock solutions of the antiseptics under test were those employed in clinical endodontics. By diluting these to strengths at which the cytotoxic effect was obtained it was possible to compare the medicaments with respect to their effect on HeLa cells. Several of the medicaments tested had thus a higher toxic effect than phenol, which was used as a reference. Comparison of the medicaments disclosed large differences between their cytotoxic effect.

An antiseptic should not irritate the tissue, and at the same time it should have a good antibacterial effect. For a more detailed discussion and comparison of the properties of the various medicaments an *in vitro* study was therefore performed in which their antimicrobial effect was examined in an environment similar to that for the cytotoxic study (*Spångberg & Engström,* 1966).

SUMMARY

A study of the cytotoxic effect of various root canal antiseptics on HeLa cells showed that several of them had a stronger such effect than phenol. Comparison between the medicaments disclosed wide differences between them as regards their cytotoxic effect.

RÉSUMÉ

ETUDES SUR LES MÉDICAMENTS POUR TRAITEMENTS DES CANAUX RADICULAIRES

I. ACTION CYTOTOXIQUE DE CERTAINS ANTISEPTIQUES DES CANAUX RADICULAIRES

Une étude concernant l'action cytotoxique sur des cellules HeLa de certains antiseptiques pour traitement des canaux radiculaires a montré que, pour plusieurs de ces antiseptiques, cette action est plus forte que pour le phénol. La comparaison des divers médicaments entre eux a mis en évidence de grandes différences en ce qui concerne leur action cytotoxique.

ZUSAMMENFASSUNG

STUDIEN ÜBER WURZELKANALMEDIKAMENTEN

I. EINE CYTOTOXISCHE UNTERSUCHUNG VON WURZELKANALANTISEPTIKA

Eine Untersuchung der Zytotoxische Effekt auf Helazellen verschiedenen Wurzelkanalantiseptiken zeigten dass mehrere von diesen einen höheren Effekt als Fenol hatten. Die Untersuchungen zeigten grosse Unterschiede wenn man verschiedene Medikamente vergleicht

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