Decontamination of root canals infected with reciprocating instruments, sodium hypochlorite 2.5% and apple vinegar

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ABSTRACT

Objective: To evaluate decontamination of root canals infected with reciprocating instruments, 2.5% sodium hypochlorite and apple vinegar. **Methods:** A total of 50 single-rooted human teeth were emptied and contaminated with *Enterococcus faecalis* for 60 days. After the period of contamination, root canal preparation was performed with instruments, including: 1. WaveOne™ 40.08 (n = 10); 2. Reciproc™ 40.06 (n = 10); and 3. Unicone™ 40.06 (n = 10). Two control groups were used: positive control (n = 10) and negative control (n = 10). Half of the samples from each group were irrigated with 2.5% sodium hypochlorite, while another half was irrigated with apple cider vinegar. Subsequently, sterilized paper cup collections were made and immersed in culture medium with 7 mL of Lethen Broth and incubated at 37 °C for 48 hours in a reduced oxygen atmosphere. Bacterial growth was analyzed by turbidity of culture medium and UV spectrophotometry. Cleaning of dentine walls was analyzed by scanning electron microscopy. **Results:** All groups showed reduction of optical density of the culture medium after root canal preparation ($p < 0.05$). No sanitation strategy promoted complete elimination of the biological indicator. Complete removal of debris occurred in none of the groups, and no significant differences were found regardless of the thirds analyzed ($p > 0.05$). **Conclusion:** Tested reciprocating instruments and irrigating solutions reduced bacterial contamination of root canals infected with *E. faecalis*.

Keywords: Root canal irrigating solutions. Root canal therapy. Root canal preparation

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Introduction

Reduction of infected root canal system microbiota is essential to successful endodontic treatment. Adequate preparation of the root canal associated with the action of antimicrobial agents (irrigating solutions and intracanal medication) plays a fundamental role in the decontamination process.[1,2]

Endodontic treatment failures have been associated with permanence of bacteria in regions of isthmus or ramifications, which hinders the action of endodontic instruments and penetration of antibacterial substances.[3,4,5]

Sodium hypochlorite is a highly studied irrigating substance used by professionals around the world.[6] This substance presents efficient cleaning and disinfection capacity but, on the other hand, a cytotoxic characteristic.[7,8] Varise et al[9] have shown that organochlorine (neurotoxic) compounds are generated during contact of sodium hypochlorite with dentine and pulp. Apple cider vinegar has been studied as an alternative in the preparation of canals because results obtained when compared to other traditional irrigating solutions have been the target of recent studies.[10,11] Its antibacterial action on the root canal and removal capacity of smear layer make it an alternative as an endodontic irrigating solution.[11,12,13]

Incorporating nickel titanium rotary instruments to endodontic practice allowed for expressive advance in root canal modeling. A better quality of preparation with centralization and maintenance of the original canal configuration has been achieved with these new systems.[4,5] Continuous-motion nickel titanium rotary instruments have been evaluated for bacterial reduction potential but no instrument has been able to act on all surfaces of dentine walls during preparation nor was able to make root canals free from bacteria and endotoxins.[14,15,16]

Yared,[17] based on the concepts of balanced forces, proposed a model for root canals preparation with a unique instrument capable of promoting root canal modeling. Various reciprocating systems have been launched on the market, such as WaveOne™, Reciproc™, TF Adaptive™ and Unicone™. These instruments are manufactured with M-Wire alloy, which is developed by special heat treatment process and which presents greater flexibility and resistance to cyclic fatigue than conventional NiTi alloy.[15,16]

The behavior of new instruments with reciprocating rotation and irrigating substances used for continuous microbial control requires special attention. This study aims to evaluate decontamination of human root canals infected with *E. faecalis* during 60 days with reciprocating instruments irrigated with 2.5% sodium hypochlorite and apple cider vinegar.

Material and methods

Samples preparation

A total of 50 single-rooted human teeth, extracted for different reasons at the emergency Department of the School of Dentistry of Universidade Federal de Goiás, were used in the present study. The study began after approval from the research ethics committee of Universidade Federal de Goiás CAAE #19811113.0.0000.5083.

These teeth were packed in a vial containing 0.2% thymol solution. Afterwards, they were immersed in 5% sodium hypochlorite (Fitofarma, Lt. 20442, Goiânia, GO, Brazil) for 30 minutes to have organic tissues removed. Buccolingual and proximal radiographs were taken using periapical films to confirm the presence of a single root canal and root apex closure, absence of previous root canal treatment, root canal obliterations, internal root resorption and/or external and cracks and fractures. Teeth were opened and emptied to apical zero with a K-Flexofile #15 (Dentsply Maillefer, Ballaigues, Switzerland), as evinced by direct visualization of the file point in the apical foramen. Anatomical diameter corresponding to K-Flexofile #35 was determined.

After initial opening and emptying procedure, the crowns were sectioned with an Endo-Z multilayer drill bit (Dentsply Maillefer, Ballaigues, Switzerland) at high rotation, with an angle of 90° to the long axis of the tooth. Root lengths were standardized at 16 mm. Subsequently, specimens were autoclaved for 30 minutes at 120 °C.

Experimental strategy

For bacterial contamination of root canals, a strain of *E. faecalis* (ACTCC 29212) inoculated in 7 mL of brain heart infusion broth (BHI; Difco Laboratories, Detroit, MI, USA) was used and incubated at 37 °C for 24 hours. The bacterial inoculum was prepared at a final concentration of approximately

3 ×10^8 cells mL-1 (McFarland turbidity scale 1). For contamination of teeth, 5 mL of sterilized BHI was mixed with 5 mL of bacterial suspension. Experimental groups were inoculated with *E. faecalis* for 60 days, repeated every 72 hours, using pure culture with 24 hours of preparation and adjusted to standard 1 of McFarland. Specimens were kept in a microbiological oven at 37 °C.

Bacterial collections were made with paper points held in the root canal for three minutes and subsequently immersed in 7 mL of BHI added with Tween 80 and sodium thiosulfate neutralizers (PA, Laboratório Art, Campinas, SP, Brazil), followed by incubation at 37 °C for 48 hours to have bacterial growth verified. Specimens were removed from the platform for the evaluation process.

Samples were randomly divided into five groups (n = 10), three of which were experimental and two were control groups. Group 1. WaveOne™ - introduced into the root canal by reciprocating movement with 40.08-diameter instrument (Dentsply Maillefer, Ballaigues, Switzerland); Group 2. Reciproc™ - introduced into the root canal by reciprocating movement with the 40.06-diameter instrument (VDW Silver, Munich, Germany); Group 3. Unicone™ - introduced into the root canal by reciprocating movement with 40.06-diameter instrument L25 (Medin, Nove Mesto in Morave, Czech Republic); Group 4. - Positive control; and Group 5. - Negative control.

In each newly prepared experimental group, five specimens were irrigated with 2.5% sodium hypochlorite (Fitofarma, Goiânia, GO, Brazil), while five specimens were irrigated with apple vinegar (Castelo, Castelo Alimentos, Jundiaí, SP, Brazil). Conventional irrigation was performed with Ultradent 5 mL syringe and Navipoint irrigation cannula (Ultradent Products Inc. 505 West 10200, South, South Jordan, UT 84095) with a diameter of 0.30 mm positioned at 12 mm. Mechanical drive of WaveOne™, Reciproc™ and Unicone™ instruments was performed with X-SMART Plus electric motor (Dentsply Maillefer, Ballaigues, Switzerland). After root canal preparation, each specimen was dried with an point#40 absorbent paper point, and the canal was filled with 3 mL of 17% EDTA, kept under stirring with manual file for 3 minutes. During a period of 60 days of root canal contamination, ten uncontaminated specimens were allowed to incubate at 37 °C as aseptic control and ten were contaminated with *E. faecalis* under atmospheric conditions similar to those previously described .

After the irrigation process in each group, additional irrigation with 5 mL sterile distilled water was performed with a syringe. Each sample was collected using sterile paper points. The points were immersed in a test tube containing 7 mL of Letheen Broth (LB; Difco Laboratories), added with neutralizers [Letheen, Tween 80 and sodium thiosulfate (PA, Lab Art, Campinas, SP, Brazil)] at appropriate concentrations, followed by incubation at 37 °C for 48 hours in a reduced oxygen atmosphere.

After root canal preparation, bacterial growth was analyzed by culture medium turbidity, thus having the presence or absence of bacteria determined, aside from the UV spectrophotometer (Spectrophotometer Model Nova 1600 UV, Piracicaba, SP, Brazil).

Preparation for SEM analysis

Roots were prepared for analysis in scanning electron microscopy. All collections were performed under aseptic conditions. Longitudinal grooves were made along the entire root length of each group specimens, and sectioning was done in a laminar flow hood with a #24 spatula and a sterile surgical hammer.

Fragments were fixed in buffered formalin solution for one week. Subsequently, dehydration was performed in a solution of 70%, 95% and 99.5% ethanol, with two exchanges per solution, totaling 30 minutes in each solution. Drying was done at critical point (AutoSamdri-815, Tousimis Research Corporation, Rockville, Maryland, USA). Metallographic preparation of teeth was carried out for analysis under scanning electron microscope (MEV, JED, JSM, 6360LV, Tokyo, Japan) with magnification of 1600 times to have the presence of debris on dentinal surface checked (Labimic, Faculty of Physics, UFG, Goiânia, GO, Brazil). The root was measured and divided equally into cervical, middle and apical thirds, which were evaluated separately.

Three-level observers analyzed the images for evaluation of absence and presence of debris on root dentinal surface according to , cervical, middle and apical thirds, using the following classifications: Score 1. Root dentinal surface with absence of debris; Score

2. Root dentinal surface with few areas covered by debris and many visible tubules; Score 3. Root dentinal surface with many areas covered by debris and few visible tubules and Score 4. Root dentinal surface completely covered by debris[30] (Fig 1).

Statistical analysis

Two different analyses were performed: non-paired, comparing densitometry values among reciprocating instruments, irrigating solutions, and instrument combination plus solution in each collection period (initial and after preparation); and paired, comparing densitometry values between collection before and after preparation.

Initially, we tested distribution of random errors around the mean (normality) and the presence or absence of homogeneous variances by Shapiro-Wink and Lévene tests, respectively. For densitometry data before preparation and collection after preparation, no normality and homogeneity of variances ($p < 0.05$) were observed.

Kruskal-Wallis test was initially applied to compare the allocation of specimens for a test of the reciprocating instruments in the control group. To conduct the test, Mann-Whitney U-test was applied to specimens allocated to both irrigating solutions in this same group. Considering interaction instrument and irrigating solution, Kruskal-Wallis test was used.

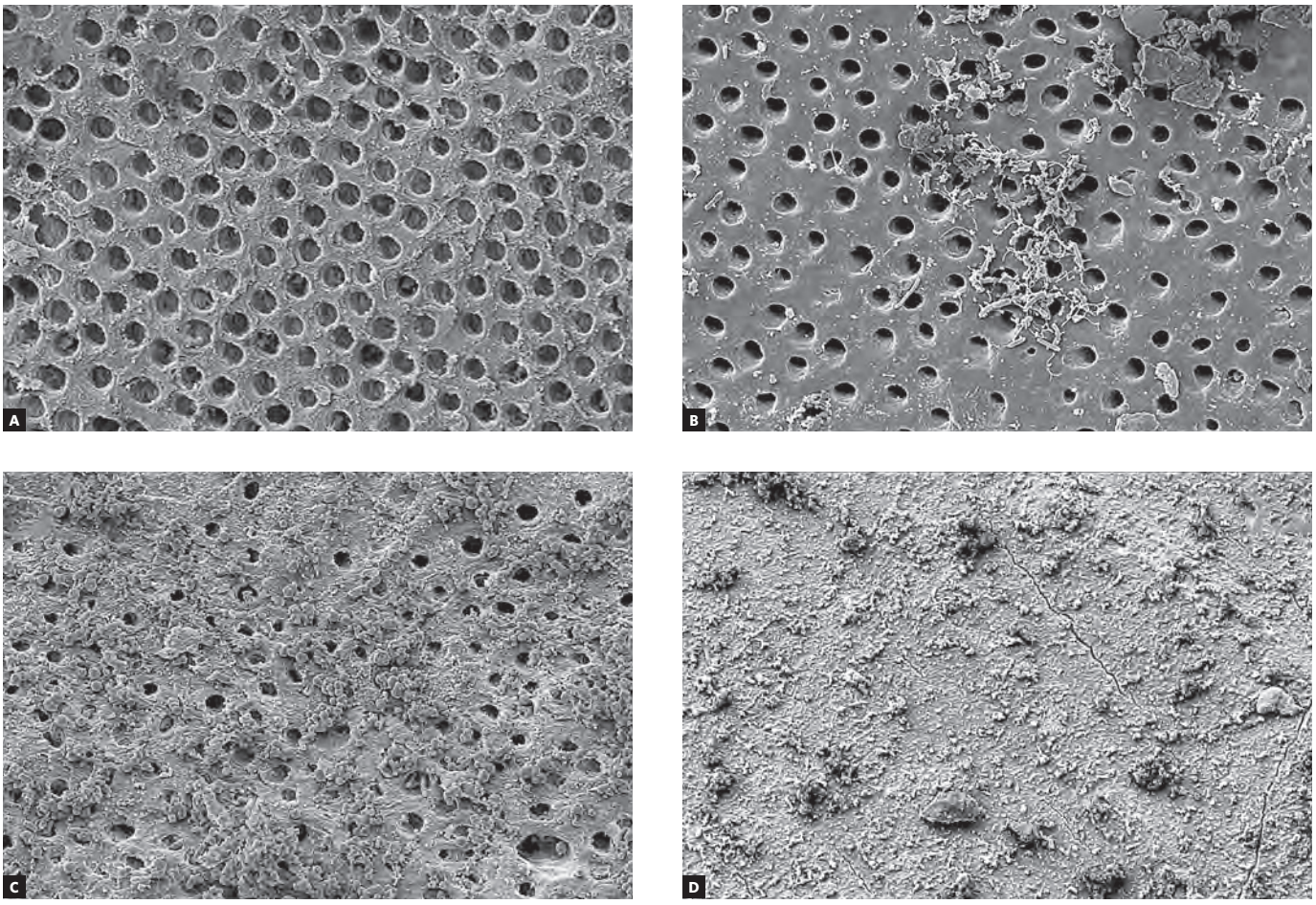

Figure 1. Classification of scanning electron microscopy images (1300x) according to absence and presence of debris on dentinal surface: **A**) Score 1 (Root dentinal surface with absence of debris); **B**) Score 2 (Root dentinal surface with areas covered by debris and many visible tubules); **C**)Score 3 (Root dentinal surface with areas covered by debris and few visible tubules; **D**) Score 4 (Root dentinal surface completely covered by debris).

In the statistical analysis of data on the removal of debris under scanning electron microscopy, Kruskal-Wallis and Mann-Whitney tests were used. The level of significance was 5%.

Results

Table 1 presents results after sanitation strategies, using reciprocating instruments WaveOne™, Reciproc™ and Unicone™, associated with irrigating solutions of 2.5% sodium hypochlorite and apple vinegar when evaluated by means of spectrophotometry. Even though all groups showed a significant reduction in optical density of the culture medium after root canal preparation ($p < 0.05$), no strategy promoted complete elimination of E. faecalis.

In all tested groups, association with 2.5% sodium hypochlorite promoted greater bacterial reduction than apple vinegar, with significant differences between them ($p < 0.001$). Reciproc™ instrument associated with 2.5% sodium hypochlorite promoted the highest percentage of bacterial reduction, while Reciproc association with apple vinegar was the one that presented the lowest.

In analysis of root surface cleaning, complete removal of debris was verified in neither group.

Discussion

Action of the reciprocating systems associated with irrigating solutions, 2.5% sodium hypochlorite and apple cider vinegar, in infected root canals, promoted bacterial reduction. In the group with 2.5% sodium hypochlorite as irrigating solution, greater decontamination of root canals was verified.

One of the determining factors of endodontic failure is post-treatment maintenance of root canal system infection, the sanitation process involving mechanical action with the objective of disaggregating = biofilm, and physicochemical effects of irrigating substances with the function of controling microbiota.[1,2,18,19]

Microorganisms organization in biofilms increases resistance of these agents to endodontic therapeutic procedures. *E. faecalis* is found in persistent endodontic infections and is able to structure itself into biofilm to deeply invade dentinal tubules and survive in microenvironments that are scarce of nutrients.[20-23] The period of contamination and the biological indicator used in the present study was 60 days, a period considered sufficient for *E. faecalis* to colonize and promote invasion of the dentinal tubules and structure a mature biofilm.[24] Other studies have employed shorter periods in biofilm formation.[25-29] The method used for bacterial collections before and after root canal preparation to verify bacterial growth or reduction in root canals infected with *E. faecalis* is a standard and widely used method for this type of analysis.[11,23,24,30]

The biological indicator *E. faecalis* has been used in several previous studies,[11,31-35] and it is considered an important bacterium in conditions of persistence of

Table 1. Mean and standard deviation of optical density (nm) of the culture medium in microbiological samples taken before and immediately after root canal preparation with reciprocating systems associated with 2.5% sodium hypochlorite and apple vinegar.

Reciprocating system	Irrigating solution	n	Mean/SD optical density of medium (nm) Collected before	Mean/SD optical density of medium (nm) Collected after
WaveOne®	2.5% sodium hypochlorite	5	0.289 + 0.089	0.007 + 0.006
	Apple vinegar	5	0.235 + 0.048	0.168 + 0.014
	Total	10	0.262 + 0.073	0.087 + 0.086
Reciproc®	2.5% sodium hypochlorite	5	0.200 + 0.058	0.001 + 0.002
	Apple vinegar	5	0.245 + 0.099	0.181 + 0.006
	Total	10	0.223 + 0.080	0.091 + 0.095
Unicorne®	2.5% sodium hypochlorite	5	0.264 + 0.098	0.034 + 0.066
	Apple vinegar	5	0.227 + 0.065	0.169 + 0.012
	Total	10	0.246 + 0.080	0.102 + 0.084

periapical lesions after endodontic treatment.[1,36] It is an optional Gram-positive coccus that tolerates low nutrient and oxygen conditions as well as high pH.[2,36,37,38]

On the other hand, several rotating NiTi instruments have been incorporated into Endodontics to improve the quality of cleaning and disinfecting the root canal system.[4] The proposal by Yared[17] on the reciprocating movement and preparation with a single instrument associated with M-Wire alloy with surface heat treatment have allowed for greater flexibility and fracture resistance when compared to conventional NiTi alloys. Thus, different methodological strategies have been continuously tested to evaluate the effectiveness of these reciprocating instruments in preserving the original root canal geometry, promoting dentine cleaning and extrusion capacity, filling material removal, cyclic fatigue resistance and their performance. However, when compared to the bacterial reduction process,[39-45] preparation simplification by means of a single instrument raises concerns about the effectiveness in promoting adequate disinfection of the root canal system.[23]

The present study results showed bacterial reduction after root canals preparation with the respective reciprocating systems: WaveOne™, Reciproc™ and Unicone™ associated with irrigating solutions of 2.5% sodium hypochlorite and apple vinegar. These results are in agreement with previous studies.[23,46,47]

Regarding root canals infected by E. faecalis, Dagna et al[46] evaluated antibacterial efficacy using NiTi (MTwo, Revo-S, and One Shape) and reciprocating (Reciproc) rotary instruments under irrigation with 5% sodium hypochlorite and 17% EDTA. All techniques were efficient in reducing bacteria, the Reciproc single-use instruments being as efficient as the conventional rotary system. Nakamura et al[23] verified the effectiveness of three techniques of manual instruments (K-File), continuous rotatory (MTwo) instruments and reciprocating (Reciproc) instruments associated with 2.5% sodium hypochlorite in oval root canals infected by E. faecalis. All techniques reduced the number of microorganisms, and Reciproc was effective in reducing biofilm with E. faecalis. Machado et al[47] evaluated the influence of reciprocating instrumentation with single instrument (WaveOne, Reciproc), continuous rotatory (ProTaper, MTwo) and manual instrumentation on bacterial reduction in root canals infected with E. faecalis. Bacterial samples were collected seven days immediately after instrumentation. Bacterial count was significantly reduced after instrumentation in all groups analyzed, with no difference among reduction byreciprocating techniques, continuous rotatory and manual instrumentation. Alves et al[48] analyzed bacterial reduction by the qPCR method and microbiological culture, and noted the antibacterial effect in oval canals comparing the technique of the single instrument Reciproc™ associated with 2.5% sodium hypochlorite and the conventional rotary technique associating BioRace™ instruments with 2.5% sodium hypochlorite. Both techniques promoted bacterial reduction with no differences between instrumentation systems.

Even though root canals preparation carried out by different reciprocating systems (WaveOne™, Reciproc™ and Unicone™) was performed by only one instrument with diameters of 40.08 mm, 40.06 mm and 40.06mm, respectively; results showed they were not different from other techniques tested.[5,15,16,18,23,40,42,47,49]

This fact may have occurred by the final enlargement of the apical portion to a diameter of 400 mm achieved in the experimental groups. Thus, it should be considered that the efficacy of the root canal system sanification process is directly associated with correct determination of the working length and the apical portion's degree of enlargement. Therefore, extending the critical apical zone to biological diameters capable of optimizing the effectiveness of auxiliary chemical and intracanal medication becomes essential.[1,2,50,51]

Methods such as computed microtomography used to evaluate the action of endodontic instruments on root canal walls reveal that extensive areas may not be touched during preparation by the endodontic instrument, which indicates the importance of irrigation in cleaning and sanitizing these areas.[3,33,34] Untreated surfaces can be disinfected by the action of irrigating solutions, which may explain absence of differences among the three groups in the present study.

Selecting an ideal irrigating solution depends on its action against microbiota of the infected root canals and the biological effect on periapical tissues. Several irrigating solutions have been advocated to reduce endodontic infection and contribute to root canal disinfection.[6,11]

Sodium hypochlorite is a widely used solution in the endodontic sanitization process, as it presents fundamental properties, such as a broad spectrum of action, antimicrobial effect (against spores and viruses) and dissolution capacity of organic matter.[6,13,52] However, this dissolution power of organic matter is not selective, which means that, especially at high concentrations, this agent can dissolve both vital and necrotic remnants indistinctly, aside from the fact that it has high cytoxicity to periapical tissues in cases of inadvertent extrusion. Studies have been carried out to find other alternatives of an endodontic irrigating solution that presents better biocompatibility than sodium hypochlorite while maintaining the properties of tissue dissolution and high antibacterial power.[7,9,13]

Different irrigating solutions, such as EDTA, citric acid, maleic acid and apple cider vinegar, have been used to remove smear layer during endodontic treatment. Apple vinegar constitutes an alternative irrigating solution acting as auxiliary in the mechanical chemical preparation of root canals. Its physical-chemical properties and efficacy in the sanification process have been investigated in previous studies.[10,11]

The presence of malic acid gives apple vinegar an important characteristic of tissue tolerance. In addition, apple cider vinegar has a remarkable medicinal potential due to its high mineral content (sodium, potassium, phosphorus, magnesium, sulfur, calcium and silicon) and other elements (pectin, beta carotene, enzymes and amino acids) thatattack free radicals contributing to the immune system.[10,11]

Regarding sanitation strategies, 2.5% sodium hypochlorite solution proved to be more effective than apple vinegar in bacterial reduction in infected dentinE. Analysis of dentine wall cleaning after the use of irrigating solutions and reciprocating systems revealed scanning electron microscopy images showed that the walls of different thirds of the root canal presented debris. Data analysis revealed no statistically significant differences among groups, irrespective of the irrigating solution and the reciprocating instrument, or their combination ($p > 0.05$).

Data obtained from the bacterial count should be interpreted with caution, as bacterial collection occurs only in the main canal, which allows detection only of the planktonic bacteria, not reaching those located in the irregularities of the root canal and deep parts of the dentinal derivatives.[23,35,37]

Correct sanitation of infected root canal system remains a real and permanent challenge. In the same way that minimal bacterial volume remaining within the root canal - necessary to promote periapical tissues repair or to maintain its health - is still unknown. Further studies with other methods and additional clinical trials should be performed to evaluate the effects of contemporary irrigation instrumentation techniques and protocols for endodontic infection.

Conclusion

Tested reciprocating instruments and irrigating solutions reduced bacterial contamination of root canals infected with E. faecalis.

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