

Effects of peritoneal lavage and dry cleaning on bacterial translocation in a model of peritonitis developed using cecal ligation and puncture

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ABSTRACT

BACKGROUND: Currently, all progress in diagnostic techniques, surgical techniques, antibiotherapy, and intensive care units is accompanied by a decrease in the mortality due to severe secondary peritonitis; however, the rate is still unacceptably high. To remove the source of peritonitis, a surgeon has several options, such as closure, exclusion, and resection, depending on the preference of the surgeon and the condition of the patient. The aim of this study is to determine the rates of bacterial translocation by comparing the dry cleaning method (gauze squeezed with saline) and peritoneal lavage method (cleaning with saline), which are among the peritoneal cleaning methods.

METHODS: A total of 64 rats were studied as sham, control, dry cleaning, and saline cleaning groups. Only laparotomy was performed in the sham group, and cecal ligation puncture was performed in the control group. After ligation puncture operations in the other two groups, one of them was subjected to dry cleaning and the other to isotonic cleaning. The samples obtained from the liver, spleen, and mesothelium were sacrificed and cultured under aerobic and anaerobic environments.

RESULTS: There was no significant difference in the anaerobic bacterial counts, although there was a significant difference in the results of the aerobic bacterial counts in liver, spleen, and mesothelium samples on comparing the dry cleaning and saline cleaning groups.

CONCLUSION: According to our study, the cleaning of intraabdominal infections with dry gauze is more effective than the cleaning with physiological saline for the elimination of aerobic bacteria. There is no difference observed with respect to the anaerobic bacterial counts.

Keywords: Intraabdominal infection; peritoneal lavage; peritonitis.

INTRODUCTION

Peritonitis is the inflammation of the peritoneal cavity. A form of peritonitis more commonly encountered by surgeons is secondary peritonitis, which is defined as the spillage of the intestinal content into the peritoneal cavity due to impaired integrity of the gastrointestinal tract.^[1]

At present, all progress in diagnostic techniques, surgical techniques, antibiotherapy, and intensive care units is accompanied by a decrease in the mortality due to severe secondary peritonitis, the rate of which is still unacceptably high. The treatment of peritonitis comprises both surgical and sup-

portive therapy. The most important rule for the success of treatment is to conduct the surgery as early as possible to prevent the release of bacteria and helper pathogens into the peritoneal cavity.

Bacterial intestinal translocation occurs when the gastrointestinal microflora passes through the lamina propria into the local mesenteric lymph nodes and then into other organs (liver, spleen, etc.).^[2] The enteric bacteria can then spread throughout the body through the systemic circulation and cause death as a result of sepsis, shock, and multi-organ failure. Therefore, surgical treatment of secondary peritonitis should be based on the control of the infection site, reduc-

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tion of contamination, and prevention of recurrent infections. To remove the source of peritonitis, the surgeon has several options, such as closure, exclusion, and resection, depending on the preference of the surgeon and the clinical condition of the patient. However, the resection of the infiltrated tissue is considered to be the best method if it is possible.^[1,3]

The second target in the surgical treatment of severe peritonitis is the removal of all necrotic and purulent material from the abdominal cavity. Although it is very popular among surgeons, the effect of intraoperative peritoneal lavage has not been found to be sufficient so far.^[4]

The aim of this study was to determine the rates of bacterial translocation by comparing two of the intraperitoneal cleaning methods: dry cleaning (with gauze made wet by saline and squeezed later) and cleaning with saline.

MATERIALS AND METHODS

This study was conducted on 64 female, 12–14-week-old Wistar albino rats weighing 190–250 g. These rats were provided with free food and water and kept at room temperature prior to the study. These 64 adult rats were randomly divided into four groups (n=16). The rats were obtained from Süleyman Demirel University Experimental Study and Experimental Animal Laboratory. The study was initiated after obtaining the Experimental Animals Research Ethics Board Approval. All procedures were applied to the rats under general anesthesia performed by administering 90 mg/kg of ketamine hydrochloride and xylazine into the peritoneum. Rats were not given any food and drink for 14 hours before the surgery.

Group 1 (Sham Group)

Peritoneal slides were obtained from this group after laparotomy. After 48 hours, the second laparotomy was performed and liver, spleen, and mesothelium tissue samples were obtained. Aerobic and anaerobic blood samples were obtained from the inferior vena cava and aorta and sacrificed.

Group 2 (Control Group)

After laparotomy, ligation was performed from 3/0 silk to the side wall of the cecum.

An 18-gauge was drilled through the end of the ligation junction with the injector tip to create perforation. After 48 hours, the abdomen was opened and liver, spleen, and mesothelium samples were obtained. Additionally, samples of aerobic and anaerobic blood cultures were obtained from the inferior vena cava and sacrificed.

Group 3 (Dry Cleaning Group)

In this group, after laparotomy, ligation was performed with 3/0 silk to the lateral wall of the cecum. An 18-gauge was drilled through the end of the ligation junction with the injec-

tor tip. After some feces were allowed to infiltrate into the abdomen, the fascia and the skin were closed. After 10 hours, the abdomen was opened again and the cecum was resected. The abdominal cavity, back of the liver and spleen, right and left paracolic areas, and pelvis were cleaned using sterile gauze. Fascia and skin were closed. After 48 hours, the liver was opened again and liver, spleen, and mesothelium tissue samples were obtained and sacrificed.

Group 4 (Saline Washed Group)

In this group, after laparotomy, ligation was performed with 3/0 silk to the side wall of the cecum. An 18-gauge was drilled through the end of the ligation junction with the injector tip. After some feces were allowed to infiltrate into the abdomen, the fascia and the skin were closed. After 10 hours, the abdomen was opened again and the cecum was resected. The abdominal cavity, back of the liver and spleen, the right and left paracolic areas, and pelvis were washed with 5 cc saline physiologically kept at room temperature (25 °C); then, the fluid was aspirated with an aspirator. This process was repeated five times. Fascia and skin were closed. After 48 hours, tissue samples were obtained and sacrificed.

Microbiological Evaluation

A total amount of 2cc-systemic blood sample from the inferior vena cava was immediately placed in blood culture bottles and taken to the microbiology laboratory for incubation. Microorganisms began to signal in 2 days. Those that were incubated and grew in Bactec 9120 BD automated blood culture system were cultured onto suitable solid media (for aerobic bacteria, blood eosin methylene blue (EMB) and chocolate agar; for anaerobic bacteria, Schadler Agar and chocolate agar) and then incubated at 37 °C for 24–72 hours. Bacteria grown on plaque were identified by BD™ BBL™ Crystal System.

Tissue specimens were weighed on a precision scale under sterile conditions, and their weight was recorded. Then, the samples to prevent deterioration during transport were placed in liquid media containing 6 ml of thiogluconate vigor and brain–heart broth, and brought to the microbiology laboratory.

The tissue samples were homogenized, and then aerobic and anaerobic microorganisms were cultured in duplicate with 0.1 ml of the blood EMB agar, chocolate agar, and Schadler media. At 37 °C, 24–72 hours of incubation was allowed in aerobic and anaerobic environment. After 24 hours, the plates were separated for colony counting and typing. Non-reproductive plaques were allowed to complete the 72-hour period. The colony counts on the reproductive plates were recorded. Gram staining was performed for aerobic and anaerobic bacterial colonies. Bacteria that were grown in aerobic medium and classified as gram negative were typed with BD™ BBL™ Crystal E/NF semi-automated identification system.

Bacteria grown in anaerobic medium were typed with BD™ BBL™ Crystal semi-automated identification system and colony counts were recorded.

The following formula was used to calculate the number of microorganisms per gram of tissue as a bacterial translocation index in tissues where colonization was detected.

Number of colonies per gram of tissue (cfu/gr) = $(N \times D \times a \times b) / W$, where the indications are as follows:

N, plate colony count

D, inoculum dilution value

a, the amount of liquid medium in which the specimen is dispensed

b, the amount of inoculum

W, specimen weight

RESULTS

The experimental group was divided into four sub-groups as: sham group, control group, dry cleaning group, and saline washed group. There were three levels of organ factor: liver, spleen, and mesothelium. Mann-Whitney U test was used for the comparison of the binary groups.

With respect to the counts of bacteria in the aerobic and anaerobic media in the liver, there was no significant difference in serum bacterial counts between the saline washed group and the dry cleaning group ($p=0.680$ and $p=0.664$, respectively) in the evaluation of microorganisms grown in aerobic and anaerobic medium in liver (Figs. 1 and 2).

A significant difference was found in both the saline and dry cleaning groups ($p=0.011$) as well as between the control group and the dry cleaning group ($p=0.013$) in the evaluation of bacterial growths in the spleen aerobic medium (Fig. 3). Statistically significant difference ($p=0.040$) was found between the control group and the dry cleaning group, whereas there was no significant difference between the control group

and the saline washed group ($p=0.746$) and the saline washed group and the dry cleaning group ($p=0.495$) in the evaluation of microorganisms grown in the spleen anaerobic medium (Fig. 4).

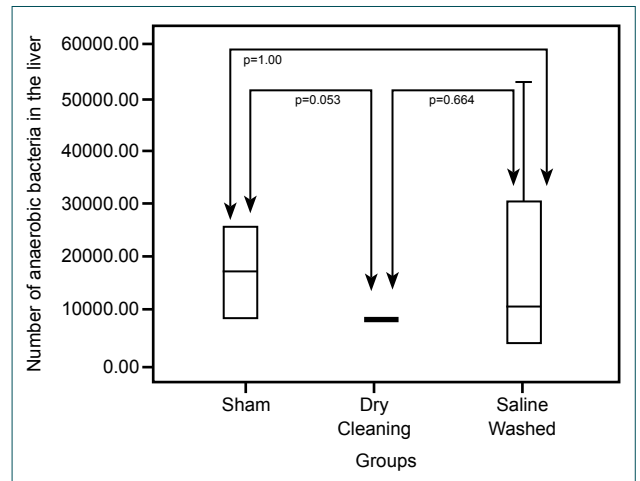


Figure 2. Statistics of bacterial count (anaerobic) in the liver.

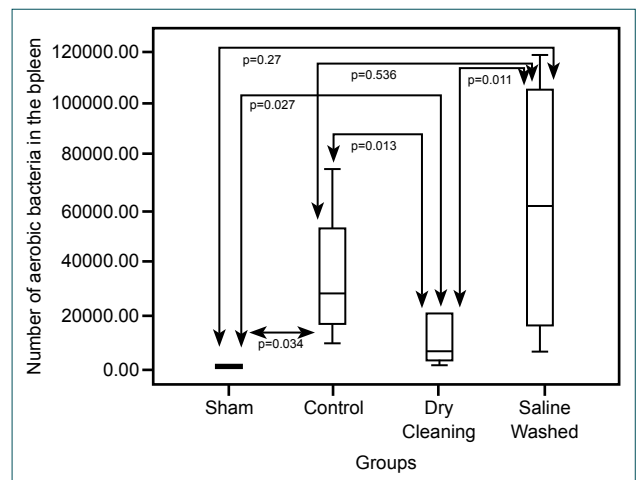


Figure 3. Statistics of bacterial count (aerobic) in the spleen.

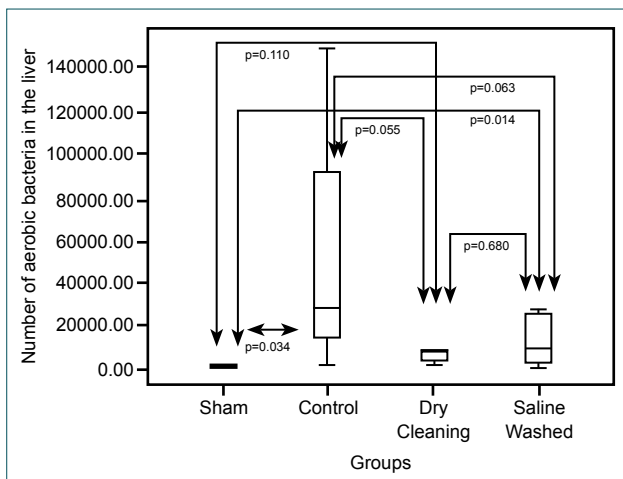


Figure 1. Statistics of bacterial count (aerobic) in the liver.

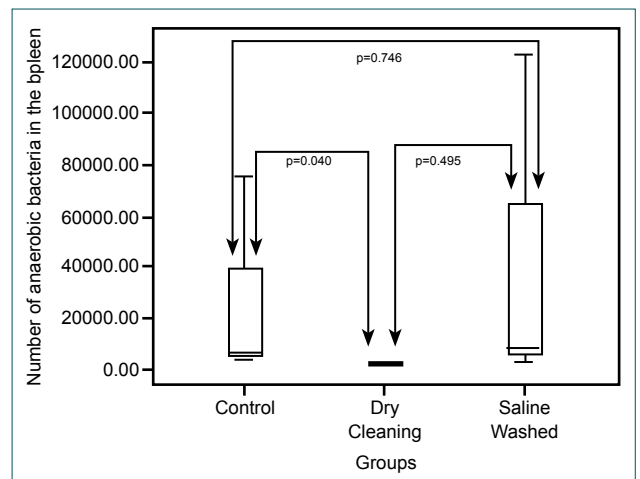


Figure 4. Statistics of bacterial count (anaerobic) in the spleen.

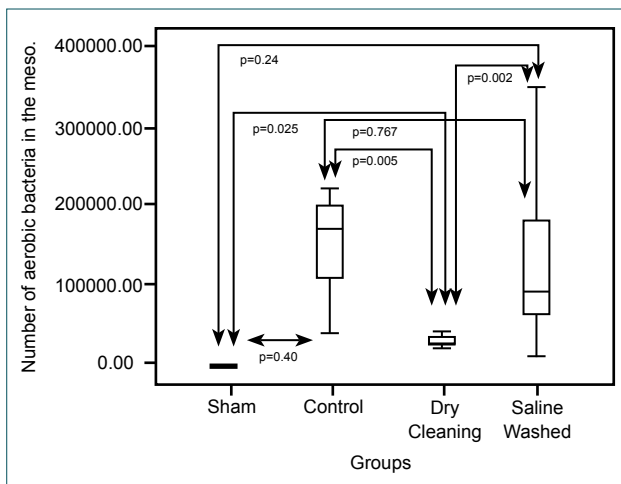


Figure 5. Statistics of bacterial count (aerobic) in the mesothelium.

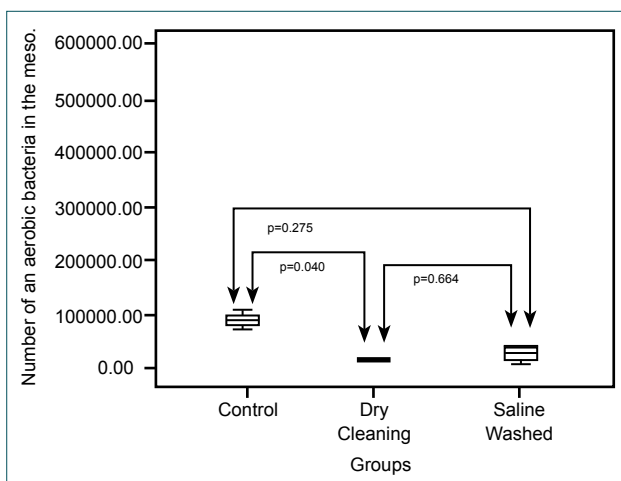


Figure 6. Statistics of bacterial count (anaerobic) in the mesothelium.

There was no significant difference between the control group and the saline washed group (Fig. 5), although there was a significant difference between the control group and dry cleaning group and between saline washed group and dry cleaning group in the evaluation of the aerobic bacterial growth at mesothelium.

When the number of anaerobic bacteria at mesothelium was evaluated, there was a significant difference between the control group and the dry cleaning group ($p=0.040$), but no significant difference was found in binary comparisons of other groups (Fig. 6).

DISCUSSION

The treatment of intraabdominal infections continues to be a problem today despite improvements in surgery. The common etiological factors are perforation of hollow organs and/or intestine and pancreatic necrosis. In our study, we used cecal ligation puncture to develop peritonitis. The cecal ligation puncture model is widely used for the modeling of sepsis and septic

shock. This model has advantages such as containing diversity of cecal microorganism, originating from the focal infection, producing septicemia, and disseminated peripheral bacterial products. Using cecal ligation puncture, it is possible to investigate chronic sepsis as well as acute sepsis and to change the severity of sepsis. Furthermore, by ligating the cecum, tissue necrosis observed in clinical sepsis after severe trauma may also be developed. Cecal ligation puncture largely resembles intraabdominal abscess formation. Another advantage of the cecal ligation puncture model is that the inoculated microbial agents are obtained from the host rather than exogenously. For the reasons mentioned above, we believe that it is a suitable model for frequently encountered situations.

In peritonitis, depending on the resistance of the organism, intraabdominal cavity abscess formation, fistula, or diffuse peritonitis may occur. The methods currently in use are improving day-by-day, but they lead to some intolerable problems. Traditionally, the importance of drainage and lavage in the treatment of intraabdominal infections has long been known.^[5,6] Currently, these two methods are widely used to control the source of infection and reduce peritoneal contamination.^[7,8] We compared the currently accepted and used lavage methods for the clinical significance of our results.

The removal of the source of infection usually requires repairing of the related organ or resection, if repair is not possible. Infectious or necrotic material must be removed from the peritoneal cavity after the removal of the source of infection. Additional materials such as foreign body, necrotic tissue, fibrin, bile, blood, and intestinal contents increase the number of bacteria when it is in the abdominal cavity and cause an increase in infection severity by deteriorating the function of macrophages and neutrophils. Because the drainage of dense contents may be difficult and insufficient,^[9-11] the peritoneal cavity should be cleaned simultaneously during the operation. There are various alternatives for this. Classically, gas compresses, dry cleaning, lavage, debridement, and postoperative irrigation are applied.

In the cases where peritoneal contamination is localized partially, dry cleaning of the area with gas compresses during operation may allow removal of most of the contaminated material from the environment, avoiding the spread to the clean peritoneal area.

Thus, it is possible to lavage the abdominal cavity more safely. There are also several studies which report that lavage does not increase bacterial spread.^[12,13]

Intraoperative lavage is a standard procedure for intraabdominal infections.

The main aim of the lavage applied during the operation is to reduce the number of bacteria as much as possible and to remove harmful foreign substances from the infiltrated area. In this way, the defense mechanisms of the organism are also

supported.^[8,9] Many of the studies in literature aimed to improve the effectiveness of currently used lavage methods by changing the content of the liquid. There are a few number studies comparing lavage with other methods.

Recently, high-volume intraoperative lavage has also been recommended.^[14] This process should be continued until the wash water is clean. Usually, 8–12 liters of physiological saline is sufficient. Antimicrobial agents may also be added to the lavage solution, but there is an ongoing debate about its effectiveness. The view that this is not necessary in patients who receive appropriate systemic antibiotic therapy has become more popular in recent years. It has been experimentally determined that lavage does not increase bacterial spread.^[13,14] It is also argued that intraoperative debridement should be performed in a limited as well as in a radical manner. Perforations after radical debridement have also been reported to be more frequent.^[1,3]

The presence of fibrin and platelets in the peritoneal cavity may weaken bacterial clearance by blocking the diaphragmatic lymphatic system.

It also causes the prematurity of peritoneal neutrophils and prevents phagocytosis of bacteria. The clearance of fluid, particulates, and microorganisms in the peritoneal cavity is usually through the diaphragmatic and parietal peritoneal lymphatic system.^[15,16]

Small diaphragmatic stomata are found around the mesothelial cells of the peritoneum covering the muscular part of the diaphragm. The presence of peritoneal inflammation increases the efficiency of these stomata and the mechanism of diaphragmatic clearance.^[17] Fluids and particles that cannot be absorbed through peritoneal and diaphragmatic lymphatic drainage are cleared from the peritoneal cavity through these stomata.^[18] In animal studies, it has been shown that clearance of fluids and particles with diaphragmatic lymphatic drainage is a very fast-acting mechanism.

Presence of microorganisms in the thoracic duct could be demonstrated at 6 minutes after intraperitoneal injection of microorganisms, but it is only possible to isolate the caval system at the 12th minute. The other mechanism of clearance is via the peritoneal macrophages. Dunn et al.^[19] demonstrated that half of the intraperitoneal bacteria were physically cleared by diaphragmatic lymphatic drainage and the other half underwent phagocytosis by the peritoneal macrophages. These two effective mechanisms are the primary mechanisms of clearance after bacterial contamination. If these mechanisms are inadequate, the peritoneal neutrophil concentration and activity increases as an inflammatory response that targets to eliminate or localize the infection.

Experimental models in the study of Zaleznik et al.^[20] have shown that microorganisms frequently isolated from intraab-

dominal infections such as *Enterobacteriaceae* and *Bacteroides fragilis* have the ability to adhere to the mesothelial surfaces and that these organisms cannot be removed from the peritoneum by peritoneal lavage or via other cleaning methods. Another study reported that bacteria still remain in the mesothelial surface after extended peritoneal lavage.^[21] Abbasoglu et al.^[22] found that intraoperative peritoneal lavage increases survival in experimental peritonitis cases. But it has been shown that performing it intermittently in the postoperative period does not affect the survival time. In addition, it was also shown that bactericidal activity of peritoneal fluid decreases in the same period due to lavage, and it recovers after just 4 hours. Another study by Abbasoglu et al.^[22] has shown that the effect of intraperitoneal povidoneiodine on the peritoneal defense mechanisms is due to toxicity and that 1% povidone iodine solution does not disrupt the local defense mechanisms of the peritoneum. The addition of antibiotics or antiseptics to irrigation solutions has shown to have a positive effect on the process of intraabdominal infections.^[23]

According to the data obtained from this study, it was found that intraoperative dry cleaning of the abdominal cavity reduced the duration of stay in surgical clinics and the number of complications. Also, in terms of drainage requirement in the surgical treatment of intraabdominal infections, it was observed that intraoperative dry cleaning provided significantly better results by decreasing the bacterial translocation rates compared to saline washing.

Conflict of interest: None declared.

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DENEYSSEL ÇALIŞMA - ÖZET

Çekal bağlama ve delmeyle oluşturulan peritonit modelinde periton lavajı ve kuru temizliğin bakteri translokasyonuna etkisi

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AMAÇ: Günümüzde, tanı yöntemlerindeki, cerrahi tekniklerdeki, antibiyoterapi ve yoğun bakım ünitelerindeki ilerlemeler, şiddetli sekonder peritonitin mortalitesini azaltmasına rağmen, mortalite hala kabul edilemeyecek kadar yüksektir. Peritonit kaynağını ortadan kaldırmak için cerrahın kapatma, dışı alma ve rezeksiyon gibi çeşitli seçenekleri vardır. Bu yöntemlerin hangisinin uygulanacağı cerrahın tercihi ve hastanın durumuna bağlıdır. Bu çalışmanın amacı peritoneal temizleme yöntemleri arasında yer alan kuru temizleme (izotonikle ıslatılıp sıkılmış gazlı bezler=kuru temizlik) ile izotonikle peritoneal lavaj yöntemini karşılaştırılarak bakteriyel translokasyon oranlarını belirlemektir.

GEREÇ VE YÖNTEM: Sham, kontrol, kuru temizlik ve izotonikle temizlik grubu olarak toplam 64 sıçan üzerinde çalışma yapıldı. Sham grubunda sadece laparotomi, kontrol grubuna çekal bağlama ve delme işlemi uygulandı. Diğer iki gruba bağlama ve delme işlemi sonrası birinde kuru temizlik diğerinde izotonikle temizleme işlemi yapıldı. Sakrifikasyon sonrası karaciğer, dalak ve mezodan elde edilen örnekler aerobik ve anaerobik ortamlarda kültüre alındı.

BULGULAR: Karaciğer, dalak ve mezo örneklerinin kültür sonuçlarında aerobik bakteri ölçümlerinde kuru temizlikle izotonik grubu arasındaki değerlendirmede anlamlı fark bulunmasına rağmen anaerobik bakterilerde ise anlamlı fark saptanmadı.

TARTIŞMA: Yaptığımız çalışmaya göre karıncı enfeksiyonların tedavisinde, karın temizliği açısından ıslatılıp sıkılmış gazlı bezle yapılan temizlik serum fizyolojikle yapılan temizliğe göre aerobik bakteriler açısından daha efektif olduğu söylenebilir. Anaerobik bakteriler açısından her iki yöntem arasında fark görülememektedir.

Anahtar sözcükler: İntraabdominal enfeksiyon; peritoneal lavaj; peritonit.

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