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The Effect of Infusion Tubing Material and Flow Rate on the Viscosity and Turbidity of Human Serum Albumin

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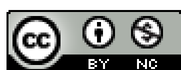

ABSTRACT

Introduction
Human Serum Albumin (HSA) is a therapeutic protein widely used in intravenous administration. Due to its structural sensitivity, HSA is vulnerable to physical instabilities such as denaturation and aggregation, potentially compromising its therapeutic efficacy. Infusion-related factors, including tubing material and flow rate, are suspected to contribute to this degradation, yet remain underexplored in routine clinical practice.
Objective
This study aimed to investigate the impact of infusion tubing materials and flow rates on the physical stability of HSA, as indicated by changes in viscosity and turbidity.
Methods
Commercially available 20% HSA solutions were passed through polyvinyl chloride (PVC) and acrylonitrile butadiene styrene (ABS) tubing at flow rates of 2 and 3 mL/min. No biochemical assays were conducted, as the evaluation focused solely on physical parameters. Viscosity was measured using an Ostwald viscometer, and turbidity was assessed using UV-Vis spectrophotometry at 350 nm and 600 nm. All experiments were conducted in triplicate, and statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test, with $p < 0.05$ considered statistically significant.
Results
Results indicated that interaction with tubing materials increased turbidity and decreased viscosity of HSA. Although both ABS and PVC altered these parameters, no statistically significant difference was observed between the two materials. Higher flow rates led to more pronounced changes in viscosity than turbidity, suggesting shear-related structural effects. Turbidity measurements at 600 nm proved more sensitive than at 350 nm. The combined use of turbidity and viscosity offers a practical, non-destructive screening method for evaluating protein stability during infusion.
Key words: *Human Serum Albumin*, Viscosity, Turbidity, Infusion Tubing

1. INTRODUCTION

Therapeutic proteins and peptides are biomolecules engineered through biotechnology to treat various diseases. These molecules function by mimicking or modifying the biological activities of endogenous proteins. Compared to small-molecule drugs, therapeutic proteins offer the advantages of high affinity and specificity toward biological targets, thereby generally resulting in fewer side effects (Akash et al., 2015; Kumar et al., 2024). Nevertheless, the stability of therapeutic proteins remains a significant challenge in both formulation and administration. Proteins are inherently labile and susceptible to degradation through chemical or physical pathways. One critical form of physical degradation is aggregation, wherein protein molecules form agglomerates that can diminish biological activity, increase the risk of immunogenicity, and ultimately lead to therapeutic failure (Devkate et al., 2016; Kopp et al., 2023).

Physical parameters such as viscosity and turbidity are widely employed as indirect indicators of protein stability in pharmaceutical preparations. An increase in viscosity may suggest the presence of intermolecular interactions or early-stage aggregation, while a rise in turbidity reflects the formation of aggregates or submicron particles in solution (Filipe et al., 2010; Z ¨olla et al., 2012). Conversely, a decrease in viscosity may indicate structural damage to the

protein, such as denaturation or fragmentation, which disrupts intermolecular interactions and the protein's native conformation (Falconer, 2019; Spalthoff, 2013).

Human Serum Albumin (HSA) is one of the most widely used therapeutic proteins for treating hypoalbuminemia. HSA preparations are available in various concentrations (5%, 20%, 25%) and are commonly administered through intravenous infusion. The physical stability of HSA during administration is critical, especially given clinical reports documenting cases of reduced patient albumin levels post-infusion, which may be attributed not only to the patients' clinical conditions but also to protein degradation during the infusion process (Amalia, 2021; Suharjono et al., 2016).

Aromatic amino acids such as tyrosine, tryptophan, and phenylalanine are commonly involved in protein degradation processes. These residues are particularly susceptible to oxidation and conformational changes, especially during physical stress such as agitation, exposure to light, or contact with synthetic infusion materials. Their structural sensitivity makes them important indicators of protein instability, including in Human Serum Albumin formulations (Belinskaia et al., 2020; Shah et al., 2020).

Factors such as protein interaction with the surfaces of infusion packaging materials—including polyvinyl chloride (PVC), polypropylene (PP), silicone, and acrylic butadiene styrene (ABS)—as well as infusion flow rates, can induce mechanical stress, denaturation, and aggregate formation (Deiringer et al., 2022; Majumdar et al., 2011). Additionally, contaminants such as microplastics from synthetic polymers have been reported to disrupt protein structure, particularly through adsorptive surface interactions (Ju et al., 2020; Rajendran et al., 2022).

A systematic investigation is warranted considering the high cost of HSA, its crucial clinical efficacy, and the limited availability of local data regarding HSA's physical stability during clinical practice in Indonesia. This study thus aims to assess the effects of viscosity and turbidity changes on HSA stability under varying infusion tubing materials and flow rates.

2. METHODS

Research Design

This study was a laboratory-based experimental investigation conducted at the Laboratory of Physical Pharmacy and Biopharmaceutics, Unit III, Faculty of Pharmacy, Gadjah Mada University. The experimental design was structured to simulate the infusion flow conditions encountered in clinical practice. The study consisted of three main phases: sample preparation, treatment of samples using different combinations of infusion tubing materials and flow rates, and physical stability analysis of the Human Serum Albumin (HSA) solution.

Instruments and Materials

The instruments used included an Ostwald viscometer, UV-Vis spectrophotometer (Thermo Scientific™ Genesys™), electronic balance (Ohaus® Pioneer™), stopwatch (ZSD - 808), refrigerator (Sharp), 30 mL vials, sterile syringes (Disposable Syringe Onemed), infusion tubing made of polyvinyl chloride (PVC) (Disposable Infusion Set Onemed) and acrylic butadiene styrene (ABS) (Disposable Infusion Set GEA), portable infusion stands, micropipettes (Dragon Lab), pH meter (HI2211 pH/ORP Meter Hanna Instruments), aluminum foil, plastic wrap, tissue papers, and standard laboratory glassware (Iwaki, Pyrex, Herma). Materials used in the study comprised 20% Human Serum Albumin (Plasbumin® Grifols), Water for Injection (PT Ikapharmindo Putramas), and 0.9% NaCl solution (PT Emjebe Pharma) as the solvent and blank.

Sample Preparation

Aseptically, 20% HSA solution was drawn using sterile syringes and transferred into 30 mL vials. The solution was then infused through two different types of tubing (PVC and ABS) at 2 mL/min flow rates and 3 mL/min. Upon completion of infusion, the solutions were collected into sterile vials for subsequent analysis. Untreated samples served as negative controls.

Determination of HSA Viscosity

Viscosity measurements were performed using an Ostwald viscometer. A volume of 15 mL of each sample was loaded into the viscometer, and the flow time was recorded with a stopwatch for each replicate. The solution's viscosity was calculated based on the Hagen–Poiseuille equation, using the flow time as the primary parameter. All experimental conditions were conducted in triplicate.

Turbidity Analysis

Turbidity testing was carried out using ultraviolet-visible spectrophotometry at 350 nm and 600 nm, following the method described by Siddiqui and Naeem (2018). The decrease in light intensity due to particle scattering in the solution was calculated as the turbidity value (τ) using the formula:

$\tau = -\ln(I/I_0)$

Where I represents the transmitted light intensity through the sample, and I_0 represents the transmitted light intensity through the blank (0.9% NaCl solution).

Assay testing to evaluate the concentration or biological activity of Human Serum Albumin (HSA) was not conducted in this study. The scope of this research was limited to physical stability parameters, namely viscosity and turbidity, to simulate the infusion process conditions. This constitutes a limitation in the evaluation of degradation, and further studies involving quantitative assays are recommended to complement these findings.

Data Analysis

Viscosity and turbidity measurement data were analyzed using GraphPad Prism software. One-way analysis of variance (ANOVA) was employed to evaluate the effects of tubing material and flow rate on the physical stability of HSA. Data were presented in tables and graphs, and a p-value of <0.05 was considered statistically significant.

3. RESULTS

Interpretation of Viscosity Measurement

Viscosity is a measure of a solution's resistance to flow and is highly dependent on intermolecular interactions within the system. In therapeutic protein formulations, viscosity reflects not only the protein concentration but also the structural integrity and the extent of intermolecular association. An increase in viscosity is often associated with protein–protein interactions, such as self-association or oligomerization, whereas a decrease in viscosity may indicate fragmentation or denaturation, whereby the protein structure becomes more flexible and loses its compact three-dimensional organization. Therefore, changes in viscosity can serve as an early indicator of physical degradation in protein systems (Falconer, 2019; Jezek et al., 2011).

The results of the viscosity measurements revealed a significant decrease in HSA viscosity across all treatment groups compared to the control group. As illustrated in Figures 1, both PVC and ABS are associated with reduced HSA viscosity for both infusion rates of 2 ml/min and 3 ml/min. There are fewer changes in HSA viscosity at PVC infusion material with a flow rate of 2 ml/minute compared to 3 ml/minutes (Figure 1a and 2a). At infussion rate of 3 ml/min, both PVC and ABS provide similar degree of viscosity reduction. A reduction in viscosity in protein systems such as HSA is generally associated with the loss of tertiary structure or disruption of intermolecular interactions, leading to a more fluid solution due to decreased molecular cohesion (Falconer, 2019). This phenomenon is considered an early indicator of protein denaturation, particularly when accompanied by the formation of aggregates.

To ensure reliability, viscosity measurements were conducted in triplicate for each sample, and the mean values were reported with standard deviations. The Ostwald viscometer used has a precision suitable for detecting minor viscosity differences in dilute protein solutions. Additionally, each measurement was time-monitored using a digital stopwatch to reduce operator error. The significant differences observed ($p < 0.0001$, as shown in Figures 1a–1b) indicate that even small changes in viscosity values are statistically and potentially biologically meaningful.

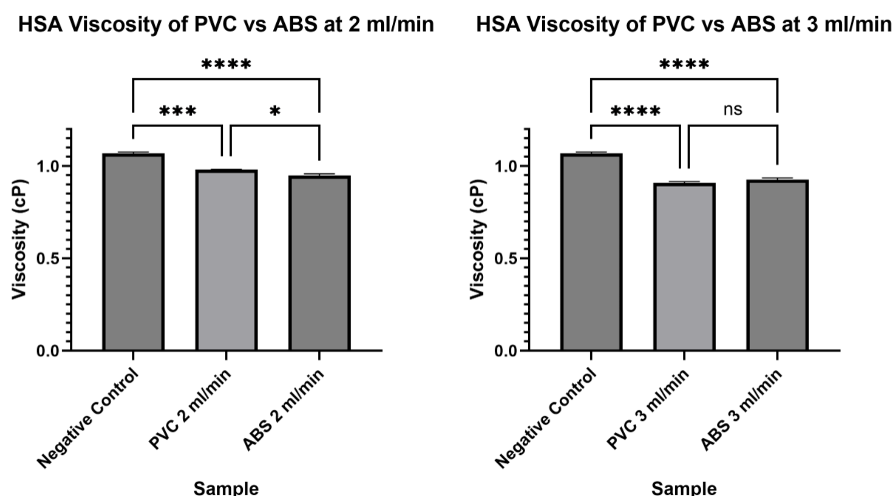

Figure 1. Comparison of viscosity measurements of samples using PVC and ABS tubing at infusion flow rates of 2 mL/min and 3 mL/min. All data represent mean ± SEM of three replicates. Data were analyzed using one-way ANOVA, followed by Tukey test. *p<0.05; ***p<0.001; **** p<0.0001

This suggests that, in addition to aggregation, protein fragmentation or unfolding may also occur as a result of surface interactions and shear forces during the infusion process (Deiringer et al., 2022). The ABS tubing exhibited a smaller decrease in viscosity compared to PVC, further indicating that the tubing material significantly influences the protein's physical stability.

Interpretation of Turbidity Analysis Results

Turbidity is an optical parameter that reflects the extent to which particles in a solution scatter incident light. In protein formulations, an increase in turbidity is typically indicative of protein aggregation, leading to the formation of large or submicron-sized particles. Such aggregates arise from physical or chemical stresses that disrupt the native conformation of proteins, rendering them more reactive and less stable (Filipe et al., 2010; Z ¨olla et al., 2012).

Turbidity measurements are commonly performed using UV-Vis spectrophotometry by assessing solution absorbance at specific wavelengths, typically between 350 and 600 nm. An elevated absorbance reading corresponds to greater light scattering, signifying the presence of aggregates. This non-destructive technique offers a sensitive means to detect early-stage aggregation without additional sample processing. Notably, Siddiqui and Naeem (2018) demonstrated that absorbance measurements at 350 nm and 600 nm are exceptionally responsive to turbidity changes in protein solutions, providing a quantitative method to monitor physical stability during storage and administration of therapeutic products (Siddiqui & Naeem, 2018).

The measurement at 350 nm was chosen based on the method of Siddiqui and Naeem (2018), which recommends this wavelength for turbidity analysis due to minimal interference from native protein absorbance. The increase in absorbance at 350 nm in our samples likely reflects light scattering by subvisible particles or early protein aggregation, rather than true absorbance by chemical groups. Hence, the significant values at 350 nm indicate increased turbidity due to particle formation during infusion.

This study measured turbidity at 600 nm and 350 nm. It revealed a significant increase in turbidity measured at 350 nm of all HSA samples after passing through PVC and ABS infusion tubing compared to the control group (Figures 2a and 2b). The difference in infusion rates showed very minimal effect on the viscosity when measured at 350 nm. Similarly, with different similar rates but different tubing, it did not give significant differences in HSA viscosity. This suggests that turbidity at 350 nm may not be sufficient to evaluate the small changes in product aggregation, although it does give evidence that the interaction of HSA with tubing material does change the product turbidity.

Since the turbidity data at 350 nm did not provide more sensitive detection of changes in product turbidity, additional turbidity evaluations were performed at 600 nm. The turbidity changes at 600 nm show a more pronounced increase in HSA turbidity (Figure 3a and 3b). The study showed that the passage of HSA solution to the PVC or ABS infusion set significantly increases the turbidity measured at 600 nm compared to those that did not have contact with it. However, the infusion rate 2 ml/min or 3 ml/min did not significantly affect the turbidity of the HSA (Figure 3a and 3b). Although not dramatic, using a higher flow rate (3 ml/min) in ABS resulted in higher turbidity than those that passed through the infusion set with a 2 ml/min rate. The material of the tested infusion set did not cause significant changes in the turbidity measured at 600 nm and 350 nm. This study suggested that HSA interactions with both PVC and ABS similarly caused the increased on-sample turbidity. The difference in infusion rate examined provides very minimal effect HSA turbidity.

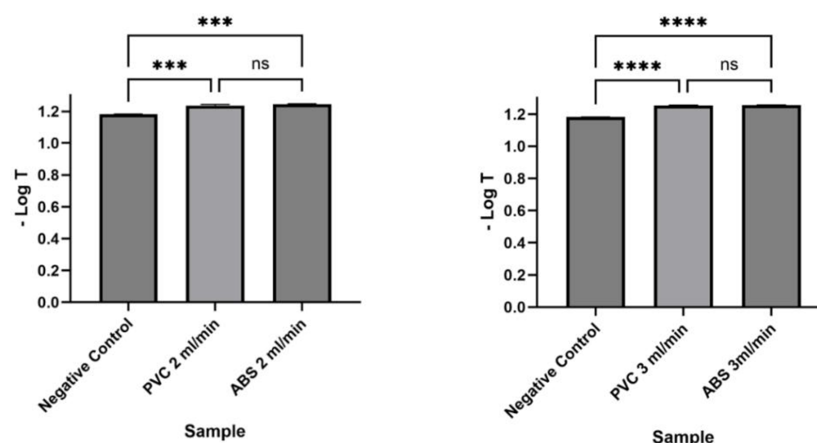

Figure 2. Comparison of turbidity analysis results of samples using PVC and ABS tubing at infusion flow rates of 2 mL/min and 3 mL/min at 350 nm. All data represent mean ± SEM of three replicates. Data were analyzed using one-way ANOVA, followed by Tukey test. ***p<0.001; **** p<0.0001

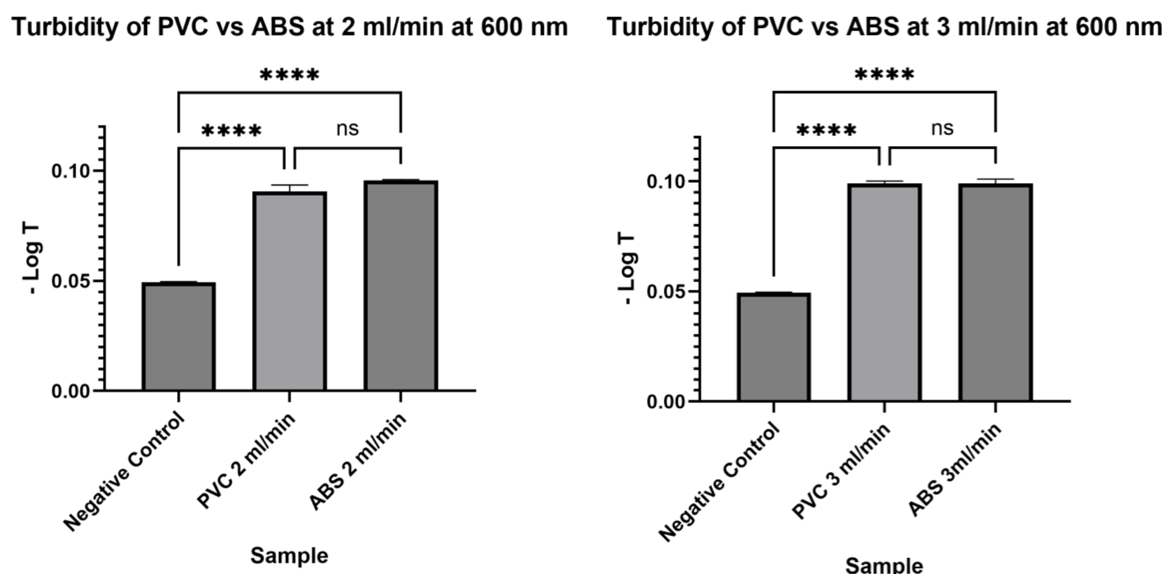

Figure 3. Comparison of turbidity analysis results of samples using PVC and ABS tubing at infusion flow rates of 2 mL/min and 3 mL/min at 600 nm. All data represent mean ± SEM of three replicates. Data were analyzed using one-way ANOVA, followed by Tukey test. ***p<0.001; **** p<0.0001

Turbidity is a commonly used indicator of aggregate particle formation resulting from protein aggregation or micro-precipitation, processes that scatter light and consequently increase the negative logarithmic absorbance value (-log T) (Z ¨olla et al., 2012). This phenomenon is strongly suspected to arise from protein interactions with the infusion tubing surface and mechanical effects induced by shear stress, particularly under high flow rates.

The hydrophobic nature of PVC facilitates non-specific interactions with proteins, promoting conformational changes and aggregate formation (Majumdar et al., 2011). In contrast, the more inert ABS tubing exhibited a relatively lower, yet still significant, increase in turbidity compared to the control group. These findings indicate that the tubing material plays a critical role in mediating the stability of therapeutic proteins during flow processes.

Correlation of Study Result and Clinical Implications

The results revealed a negative correlation between viscosity and turbidity, whereby an increase in turbidity accompanied a decrease in viscosity. This correlation supports the hypothesis that structural damage to proteins, induced by shear stress and surface interactions, not only reduces the solution's viscosity but also facilitates aggregation, as evidenced by increased turbidity (Filipe et al., 2010; Jezek et al., 2011).

These findings carry significant clinical implications, particularly concerning the selection of infusion tubing and adjusting flow rates for HSA preparations. Using more inert infusion materials, such as ABS, and adopting moderate flow rates could help minimize protein degradation, preserve therapeutic activity, and reduce the risk of patient immune responses. Moreover, the results highlight the importance of conducting physical evaluations of protein preparations within local clinical contexts, especially given the high cost associated with HSA as a therapeutic protein.

Protein Stability and Its Pharmaceutical Relevance

Proteins are complex polypeptides typically in a structured state, stabilized through intramolecular interactions and interactions between the molecules and the solvent. The ability of proteins to fold into unique conformations creates a highly diverse and dynamic chiral physicochemical environment, a feature not observed in small molecules. This characteristic enables proteins to play extensive and complex pharmacological roles in the treatment of a wide range of diseases (Rahban et al., 2023). In the pharmaceutical field, the stability of protein-based drugs is a critical aspect that must be rigorously maintained throughout formulation, storage, and distribution processes. Structural instability caused by misfolding, partial denaturation, or covalent and non-covalent modifications may significantly alter the biological characteristics and activity of therapeutic proteins (Elgamacy, 2022).

Efforts to preserve the structural integrity of therapeutic proteins and peptides have been a central focus of pharmaceutical research over the past few decades (Akbarian & Chen, 2022). One of the major challenges in developing and storing protein drugs is aggregation — a process whereby proteins associate to form clusters or aggregates. These aggregates reduce therapeutic efficacy and may also be recognized as foreign entities by the immune system, triggering immune responses and the generation of anti-drug antibodies. Protein aggregates, particularly those of micron size with altered secondary structures, are known to have a high potential for

immunogenicity (Ahmadi et al., 2015). Furthermore, interactions between proteins and the surfaces of materials, such as packaging or infusion devices, can lead to loss of biological activity and provoke immune or foreign body reactions in vivo (Marruecos et al., 2018).

Human Serum Albumin (HSA) is a small, highly water-soluble globular protein with over 500 amino acids. Common types of albumin used in healthcare include human serum albumin (~67 kDa), bovine serum albumin (~69 kDa), and ovalbumin from egg whites (~47 kDa) (Belinskaia et al., 2020). Albumin plays a critical role in maintaining plasma oncotic pressure, fluid distribution, acid-base balance regulation, and acts as an antioxidant and carrier for drugs such as digoxin, warfarin, and anti-inflammatory agents (Hutapea et al., 2023).

The conformational stability of HSA is pivotal for ensuring therapeutic success, particularly in cellular applications. Stable albumin maintains biological activities, supports cell growth, and prevents adverse immune responses. Conversely, denatured or aggregated albumin may reduce therapeutic effects and trigger an immunogenic reaction. Numerous studies have demonstrated that the conformational stability of HSA is strongly influenced by environmental conditions such as temperature, pH, excipients, and formulation additives. Thus, the careful selection of storage conditions and formulation strategies is essential for preserving HSA stability (Wynendaele et al., 2021).

Viscosity and turbidity are two important parameters commonly used to monitor the physical stability of proteins in solution. Viscosity reflects the extent of intermolecular interactions among protein molecules; a decrease in viscosity may indicate partial denaturation or fragmentation, while an increase may suggest association, oligomerization, or early aggregation (Falconer, 2019; Jezek et al., 2011). Meanwhile, turbidity is a highly sensitive indicator of aggregate formation, resulting from the scattering of light by proteins that are no longer fully soluble. Elevated turbidity values are often associated with the emergence of submicron and micron-sized particles due to mechanical, chemical, or thermal stress (Filipe et al., 2010; Z ¨olla et al., 2012). Therefore, these two parameters, when used together, provide a significant early indication of structural changes in therapeutic proteins and are widely adopted as non-destructive methods for evaluating protein stability.

Although no direct correlation assay or conformational analysis was performed to validate the stability status of HSA, alterations in viscosity and turbidity are recognized as early physicochemical indicators of protein aggregation and denaturation. These parameters were selected to simulate the effects of infusion conditions on HSA behavior in a clinical setting, with the understanding that further structural and functional analyses would be required to confirm biological degradation.

Study Limitations

This study was limited to physical parameters, namely viscosity and turbidity, assessed over a short observation period. Further evaluations, such as secondary structure analysis (e.g., Circular Dichroism) or bioactivity assays of HSA post-treatment, are necessary to provide a more comprehensive understanding. Additionally, future studies involving other materials, such as silicone or polyethylene, are recommended to broaden clinical relevance.

4. CONCLUSION

The findings of this study demonstrate that Human Serum Albumin (HSA) experiences reduced viscosity and increased turbidity when interacting with infusion tubing made from PVC or ABS. The infusion rate examined (2 ml/min and 3 ml/min) significantly reduces viscosity more than increasing turbidity. The reduced viscosity and increased turbidity of HSA upon interaction in the infusion tube seem to have a similar magnitude on both PVC and ABS materials. Therefore, both PVC and ABS infusion sets can be used to deliver HSA in the clinic, but are recommended for more moderate infusion flow rates (2 mL/min) to preserve HSA viscosity and turbidity value. Further studies are warranted to investigate in more detail the protein conformation when HSA interacts with PVC and ABS using spectroscopic method and others.

5. ACKNOWLEDGMENT

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