Stratum corneum symmetry breaking: the polarity for permeability

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Abstract

The stratum corneum (SC), as the outermost layer of the skin, plays a barrier function of the body. On the other hand, topical products, including cosmetics, must penetrate the SC and deliver ingredients to the appropriate location in the body. Thus, it is thought that there is a trade-off relationship between the barrier function and permeability of the SC. To solve this problem, we hypothesized that the SC may have polarity, or “symmetry breaking”, in permeability and attempted to further elucidate the function of the SC.

To test this hypothesis, we evaluated the permeability of water and sodium fluorescein in the direction of outside-in and inside-out by using SC derived from human abdominal skin with Franz cells. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was also used to comprehensively analyze molecular localization within the SC.

In permeability evaluation, we confirmed that there is difference in permeability depending on the direction of diffusion and the SC is not a simple homogeneous membrane but has depth-dependent permeability characteristics, that is polarity. It suggests that this is due to heterogeneous diffusivity of drugs or water within the SC. Moreover, comprehensive analysis by ToF-SIMS showed lipid-derived component localization in the upper and lower SC.

These results suggest that the SC may strategically establish spatial inside-out and outside-in barrier functions by forming changes through turnover and creating polarity in permeability, or "symmetry breaking." This clarification of the function of the SC is expected to be a breakthrough for both strengthening the barrier function and improving transdermal permeation.

Keyword

stratum corneum, permeability, ToF-SIMS, barrier function, symmetry breaking

Introduction

There are various kinds of active ingredients and base agents contained in cosmetics and topical products. These ingredients need to be delivered efficiently to their respective target sites when applied to the skin. However, the SC, the outermost layer of the skin, makes transdermal penetration of substances difficult. The SC is mainly composed corneocytes and intercellular lipids, that is often compared to bricks and mortar. The bricks are corneocytes, which are keratinocytes in the epidermis that have undergone differentiation and lost their nuclei, and the lipids that surround them fill the intercellular spaces like mortar [1]. Intercellular lipids are composed of ceramide, free fatty acids, cholesterol, etc., and form a lamellar structure in which hydrophobic and hydrophilic portions are regularly repeated [2]. These organization express a barrier function to protect the human body from water transpiration and invasion of foreign substances [3].

While this barrier function is essential for the survival of life, the transdermal absorption of ingredients is also required. In other words, a trade-off relationship exists between the permeation and the barrier function. This is a problem that needs to be resolved urgently. In order to solve this problem, we hypothesized that the SC does not have a homogeneous barrier function, but rather that there is a polarity, or "symmetry breaking", with respect to inside-out and outside-in permeability, and attempted to further elucidate the SC barrier function. The trade-off relationship can be eliminated by the polarity in term of permeability. As it can selectively prevent evaporation of water while allowing a certain amount of ingredients applied from outside the body to permeate through.

In the SC, corneocytes become enlarged and flattened according to turnover. As described in the Landman model[4] and Membrane Folding model[5], the lipids derived from lamellar granule released by enucleation of keratinocytes form a lamellar structure in the lower SC and these lipids are degraded by enzymes such as lipase and steroid sulfatase in accordance with turnover. Given that the biological system is tightly and intricately regulated, polarity resulting from depth-dependent heterogeneity seems to exist even within the SC, which is as thin as several tens of micrometers. In fact, there is a report[6] which the distribution of sodium

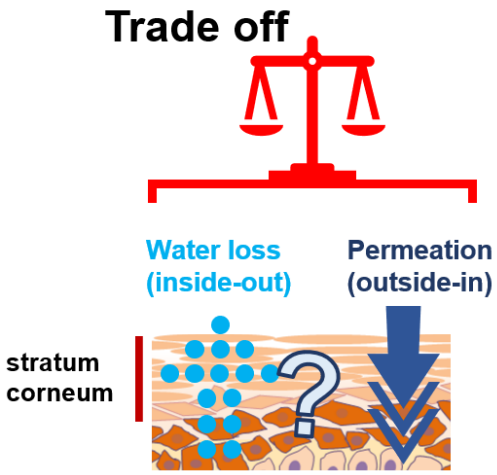

Figure 1. Trade-off relationship between permeability and barrier function and our question about SC.

ions, potassium ions, and amino acids divides the SC into three layers. However, few studies on the barrier function of the SC have focused on the directionality of its permeation. In this study, we attempted to elucidate a novel SC function called "symmetry breaking" by focusing on the directionality of its permeability.

Materials and Methods

1. Measurement of water transpiration through the SC

The SC was derived from human abdominal skin (Biopredic International inc., Saint Gregoire, France) and the Franz cell (PermeGear Inc., Hellertown,PA, USA) with a 5-mm aperture was used. The SC was stored at -80°C. After thawing, the SC was cut into approximately 15 mm squares. The inside-out transpiration was evaluated making the surface side of the SC faces upward, and the outside-in transpiration was evaluated by making the back of the SC face upward. The receiver phase was filled with 5 mL of degassed PBS(-) and acclimatized in a constant temperature and humidity chamber (32°C, 50%RH) for 30 minutes. After acclimation, the weight containing Franz cells was measured on a precision analytical balance (AP225WD; SHIMADZU inc., Kyoto, Japan) and the value was used as the starting weight (0 hr.). It was weighed at different time intervals up to 24 hours in a constant temperature and humidity chamber, and the decrease was used as the amount of water transpiration. Linear approximation was performed in the range of 3-24 hours, and the amount of water transpiration per unit time was calculated.

2. Evaluation of FLNa permeability

The outside-in permeability was evaluated making the surface side of the SC faces upward when set in the Franz cell, and the inside-out permeability was evaluated by making the back of the SC face upward. The receiver phase was filled with 5 mL of degassed PBS(-) and stirred with a stirrer during the experiment. Water at 32°C was circulated through the jacket of the Franz cell using a thermostatic bath. Sodium fluorescein (FLNa; Fujifilm Wako inc., Osaka, Japan) was dissolved in distilled water to make 1.0 mg/mL and dropped 100 μL of it into the donor phase. A volume of 150 μL of receptor fluid was collected at different time intervals up to 24 h, and an equal volume of PBS(-) solution was added to the receptor compartment. Fluorescence intensity was measured using VARIOSCAN FLASH (Thermo Fisher Scientific inc., Massachusetts, U.S.A.) at 495 nm excitation/ 550 nm measurement wavelength.

The apparent permeation parameter of FLNa was calculated from the following equation derived from the diffusion model.

$$Q = \frac{KCvD}{L}t - \frac{KCvL}{6}$$

where Q is the cumulative permeated amount, L is the thickness of the SC, Cv is the drug concentration in the donor phase, t is time, K is the apparent partition coefficient, and D is the apparent diffusion coefficient. L = 20 μm according to a previous report [7].

3. Molecular localization analysis of SC by ToF-SIMS

ToF-SIMS analysis of vertical cross sections was performed in SC. (TOF-SIMS5, primary ion source Bi; IONTOF inc., Muenster, Germany)

Results

1. Measurement of water transpiration through the SC

To evaluate the polarity of transpiration through the SC, we compared the water loss by inside-out and outside-in transpiration. As shown in **Figure 2**, the amount of loss differed depending on the direction of water transmission. It was more suppressed in the inside-out transpiration. This is consistent with the direction that prevents transpiration from inside the body.

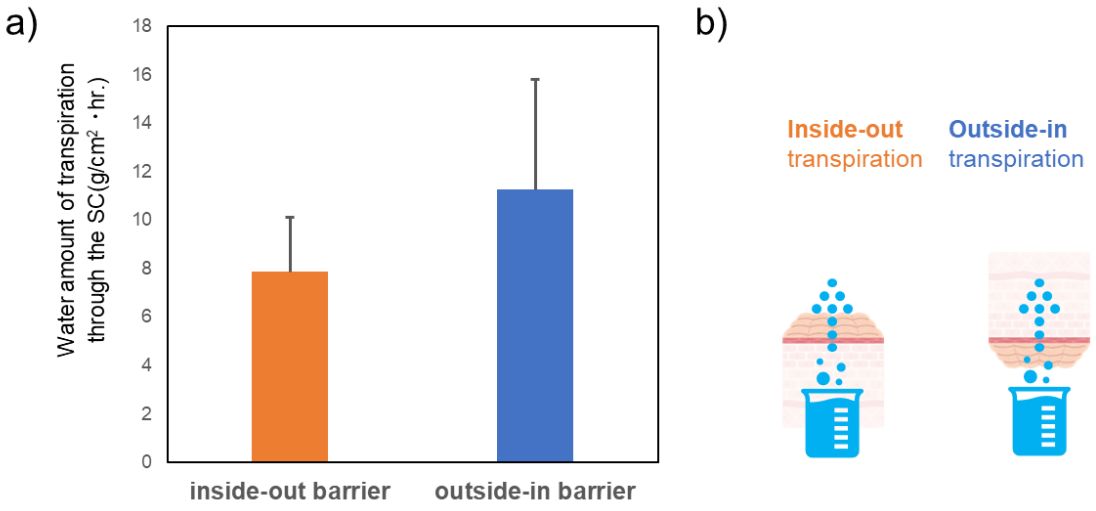

Figure 2. a) Water loss of transpiration through the SC per unit area and unit time. The experiment is performed under 32°C and 50%RH. The value is mean ± SD.

b) Experimental conditions for each legend. The inside-out transpiration was evaluated making the surface side of the SC faces upward, and the outside-in transpiration was evaluated by making the back of the SC face upward.

2. Evaluation of FLNa permeability

To evaluate the polarity of permeability of drugs through the SC, we compared the permeability of a model reagent, sodium fluorescein, from the surface of the SC (outside-in) and from the back of the SC (inside-out). If the SC was homogeneous, the permeability behavior would be consistent regardless of outside-in and inside-out, but as shown in **Figure 3**, the amount of permeated FLNa differed between the two models. Therefore, the SC seems to have the polarity in term of the permeability.

From the obtained results, a steady-state approximation (3-24 hr.) was performed and the apparent diffusion and partition coefficients were calculated using Fick's diffusion equation for inside-out and outside-in, respectively, as shown in **Table 1**.

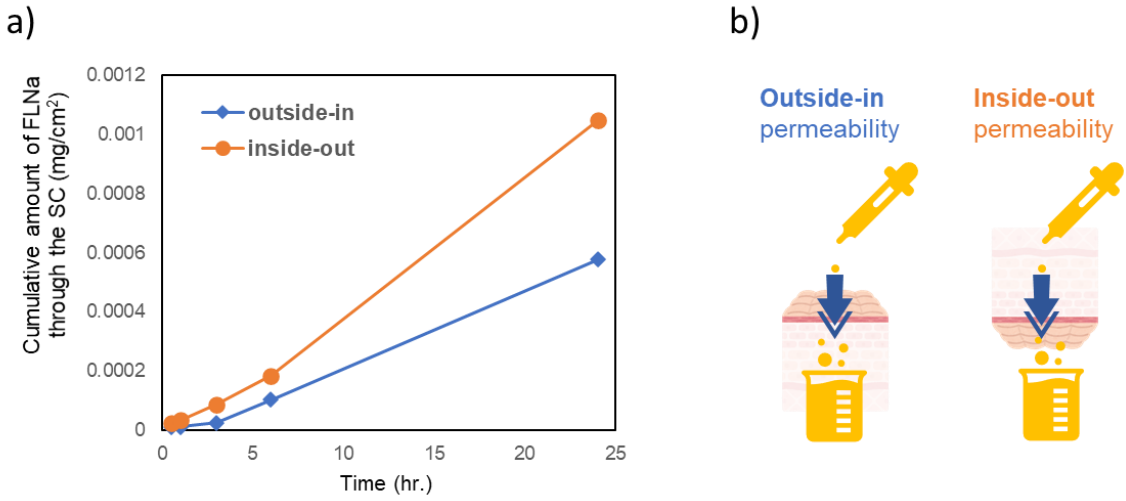

Figure 3. a) Cumulative amount of FLNa through the SC per unit area. b) Experimental condition of each legend. The outside-in transpiration was evaluated making the surface side of the SC faces upward, and the inside-out transpiration was evaluated by making the back of the SC face upward.

Table 1. Calculated permeation parameter. Where *K* is apparent partition coefficient and *D* is apparent diffusion coefficient in each model.

	Outside-in	**Inside-out**
K (-)	0.160	0.211
D (cm^2/hr.)	3.28×10^{-7}	4.39×10^{-7}

3. Molecular localization analysis of SC by ToF-SIMS

ToF-SIMS was used to analyze the molecular localization in the SC. In this study, the localization of fatty acids was observed on the surface side of the SC (**Figure 4**). In the lower part of the SC, localization of phosphoric acid, which is considered to be derived from phospholipids, was observed (**Figure 5**).

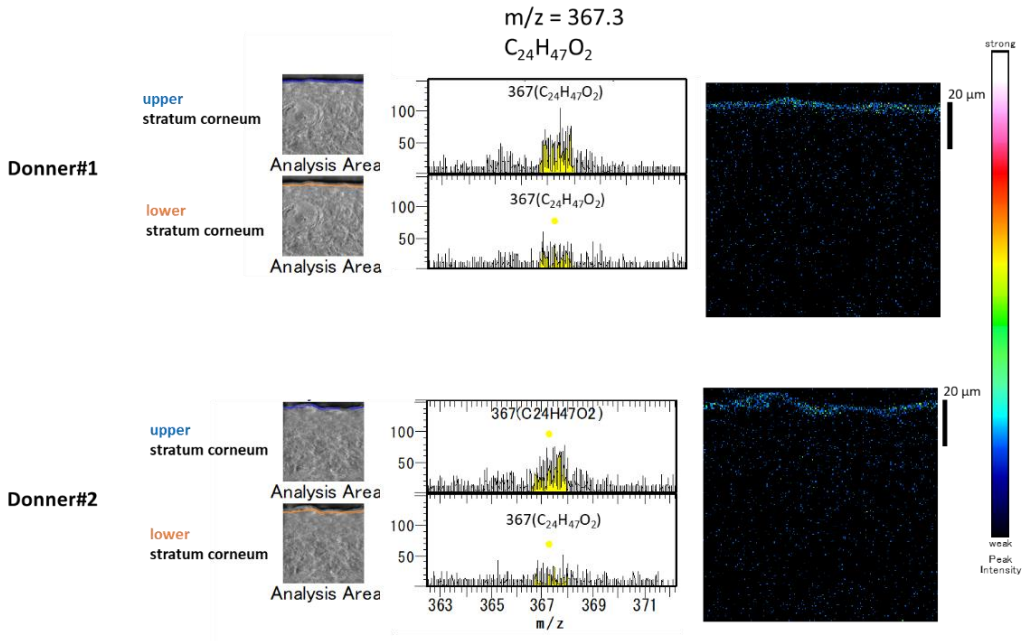

Figure 4. The localization of fatty acids analyzed by ToF-SIMS.

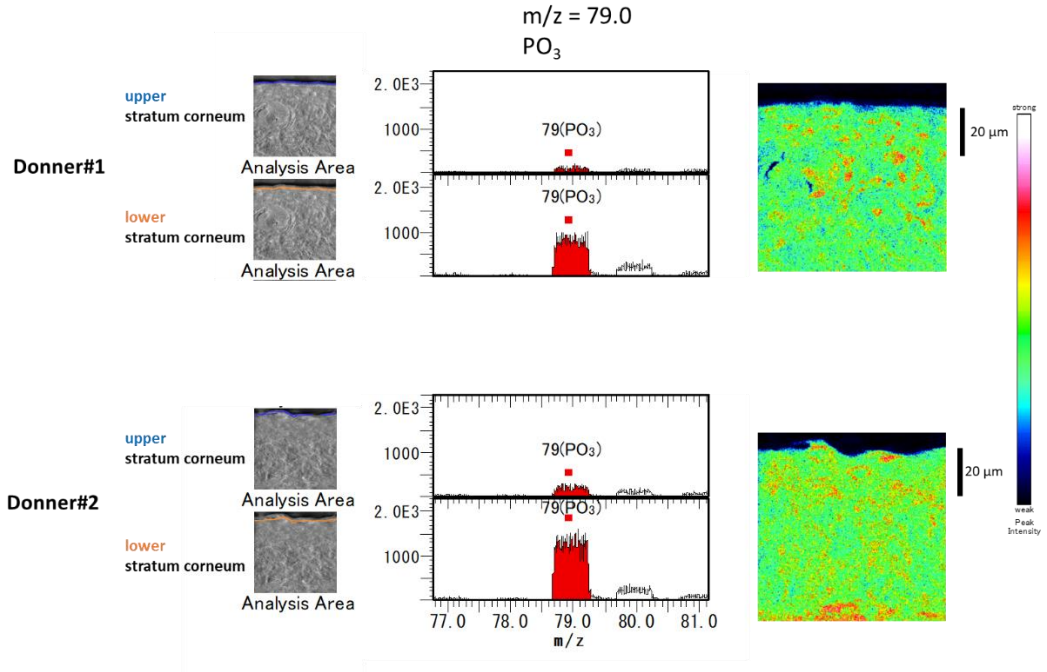

Figure 5. The localization of phosphoric acid analyzed by ToF-SIMS.

Discussion

In this study, we hypothesized that polarity, or "symmetry breaking", with respect to inside-out and outside-in permeability may exist in the SC and attempted to further elucidate the SC barrier function.

At the beginning, in the evaluation of the inside-out and outside-in permeability of water transpiration, differences were observed in the two models. In the evaluation of the permeability of FLNa, a model reagent for hydrophilic, in case of outside-in and inside-out revealed differences in two models' permeability. It is suggested the existence of polarity within the SC. The substance permeation has two pathways. The one is the unbroken SC pathway, the other is the sweat ducts or hair follicle pathway [8]. The unbroken SC pathway was further classified into the intercellular and transcellular routes [9]. The water content in the SC is about 25 wt%, and most of it is found in the corneocytes [10, 11]. However, given that intracellular water is bonding water [12] and that a small amount of water is pooled in the aqueous phase of the short lamellar lipid structure [13], intercellular lipids are can be involved in the permeation of water with transpiration. Also, since appendages such as sweat ducts and hair follicle account for only about 0.1% of the skin's surface area [8], their contribution to permeation is small. So, it is considered that intercellular lipids are involved in the FLNa permeation pathway. The intercellular lipids packing in the SC are known to be orthorhombic, hexagonal, and liquid crystalline, and it has been reported that the higher the percentage of highly ordered orthorhombic lipid phase, the lower the trans-epidermal water loss (TEWL)[14]. If the permeability of the SC is macroscopically homogeneous, the behavior of water transpiration or FLNa permeation through the SC is expected to be similar regardless of its direction. Therefore, based on the results of this study, it can be considered that depth-dependent inhomogeneity exists in the intercellular lipid packing, which may be responsible for the polarity between the inside-out and outside-in observed in this study.

The depth-dependent heterogeneity within the SC have been further discussed using a diffusion model at steady state. The diffusion model is a method to consider the pharmacokinetics of drugs in the skin, assuming that the drugs follow the mathematical model derived from Fick's law. Applying this to our study, we hypothesized a equal division model in which the SC is divided into two in the depth; upper SC and lower SC. Assuming that the diffusion coefficients in each division are D_{upper} and D_{lower}, the apparent partition coefficient in outside-in is K_{upper}, the apparent diffusion coefficient in outside-in is $D_{o\text{-}i}$, the apparent partition coefficient in inside-out is K_{lower}, and the apparent distribution coefficient in inside-out is $D_{i\text{-}o}$., their relationship is expressed by the following equation.

$$D_{\mathrm{o-i}} = \frac{2}{\frac{1}{D_{\mathrm{upper}}} + \frac{K_{\mathrm{upper}}}{D_{\mathrm{lower}}}}$$

$$D_{\mathrm{i-o}} = \frac{2}{\frac{1}{D_{\mathrm{lower}}} + \frac{K_{\mathrm{lower}}}{D_{\mathrm{upper}}}}$$

In the results obtained in this study, D_{upper} and D_{lower} are calculated as shown in **Table 2**.

Table 2. Calculated apparent diffusion coefficient in each division.

D_{upper} (cm^2/hr.)	2.96×10^{-7}
D_{lower} (cm^2/hr.)	1.80×10^{-7}

The flux of the drug in the skin is expressed as $J = -D\frac{\partial C}{\partial x}$ (Fick's first equation). In the present results, diffusion coefficients are different between the upper and lower SC. In addition, diffusion coefficient of upper region is higher than lower region. Some reports, using Electron spin resonance [15], Raman spectroscopy [16], and Fourier transform infrared spectroscopy [17], have revealed that the lower part of the SC has more tightly packing of intercellular lipids. Considering these reports, our results suggest that concentration gradient-independent differences in diffusivity are observed within the SC, and that the formulation of polarity in term of permeability within the SC is dependent on heterogeneity in diffusivity.

Comparing the inside-out and outside-in water transpiration and FLNa permeation, it is turn out that their polar directions are opposite. The reason for this difference depending on the kind of substance is currently under consideration. But it is interesting to be opposite relationship between hydrophilic model drug and water, which is indicator of barrier function of the skin. This requires further investigation.

To seek the cause of polarity in diffusivity within the SC, molecular localization was analyzed by ToF-SIMS, and the distribution of fatty acids and phospholipids was observed in the SC. Intercellular lipids are most abundant in ceramide by weight, and ceramide is degraded to fatty acids by ceramidase following turnover after forming a lamellar structure. Therefore, the localization of fatty acids observed in this study may represent the degradation products of ceramides during turnover.

It is considered that if humans lose their SC completely, we cannot survive for 24 hours

due to loss of water. In the expression of barrier function at tight junctions in the living cell layer of the epidermis, it has been reported[18] that keratinocytes strategically change their own morphology and temporarily double the barrier around the cell, allowing the cell to be replaced while maintaining only one layer of tight junction barrier. As this example shows, because barrier functions are essential for the survival of life, expression of barrier functions is precisely and intricately regulated by biological systems, and the production, packing, and degradation of intercellular lipids are no exception to this rule. In this study, we suggest that the molecular localization of ceramide in the SC during the turnover process results in heterogeneous diffusion of drugs in the skin. This is not a consequence of SC exfoliation (turnover), but a strategy of the biological system to express polarity, or "symmetry breaking", in term of permeability within the SC, which may lead to further clarification of SC function.

Conclusion

Our research has revealed that there are differences in the outside-in and inside-out permeability of the SC, even for the same substance (**Figure 6**). It is suggested that the SC forms quantitative, qualitative, and structural changes due to turnover and that it may strategically establish spatial barrier. We believe that this elucidation about function of the SC is a breakthrough for both strengthening barrier function and improving transdermal permeation.

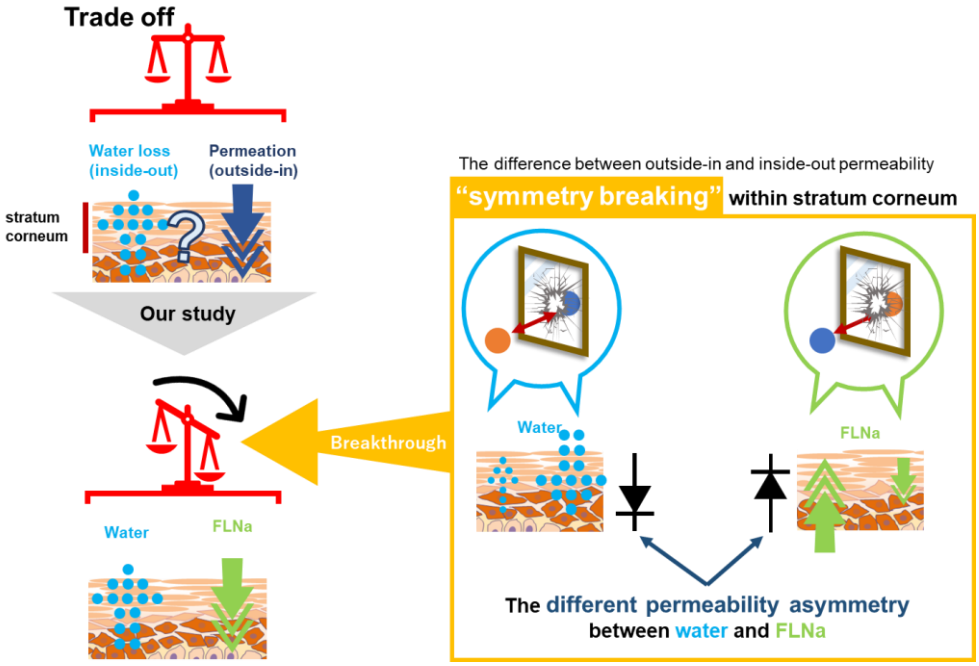

Figure 6. Our study schematic diagram

Conflict of interest Statement

NONE

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