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# Observations on Anomalous Retention in Steric Field-Flow Fractionation

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## Abstract

We discuss two anomalies that have characterized our recent work in steric field-flow fractionation (steric FFF): (a) retention has been found to vary with flow rate, contrary to simple theory; and (b) low density cells elute earlier than theory predicts. After reviewing the theory, we describe experiments in both gravitational and centrifugal fields applied to two spherical beads (microporous silica and polystyrene latex) of essentially equal sizes but different densities. The velocity dependence of retention is verified for both kinds of particles, but we find that retention depends dramatically on density as well. We speculate that these anomalous dependencies originate in a velocity-dependent lift force acting to pull the particle away from the wall. This hypothesis is supported by the observation that an increase in field strength increases retention. We conclude that a controllable field strength (as in a centrifuge) is an important asset in steric FFF, permitting increased separation speed and allowing the option of separating particles on the basis of density as well as size.

## INTRODUCTION

Recently the field-flow fractionation (FFF) technique has been extended to allow the separation of particles with dimensions in the 1 to 100 $\mu$m range (*1–3*). This modification, referred to as steric FFF, separates a complex particulate sample according to the size of its constituents by forcing larger particles through the separation channel at faster rates than smaller

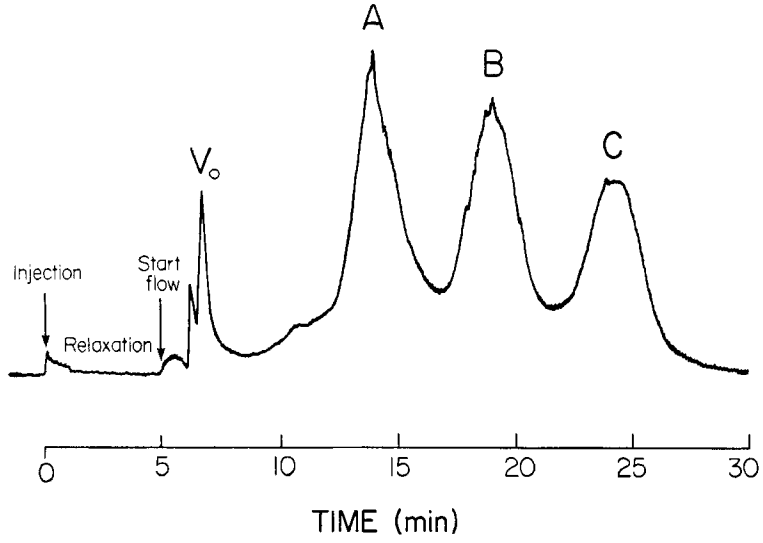


FIG. 1. Steric field-flow fractionation of silica beads. Peak A represents particles in the 10 to 14 $\mu$m size range; B, those of 7.5 $\mu$m average diameter; and C, 5.6 $\mu$m diameter. The carrier, 0.01 *M* aqueous ammonia, was flowing at 39.1 ml/hr.



ones. The resulting fractograms demonstrate the high resolving power and relatively high speed of this method (see Fig. 1). Since the elution position of sample silica beads relates linearly to particle size at a given velocity (Fig. 2), steric FFF can be used to determine size characteristics of particulate samples. This has been illustrated using a wide range of particles commonly employed as chromatographic supports (*4*). The bead dimensions determined through this fractionation procedure correlated well in most cases with data from microscopic observations.

Results presented in Ref. *4* give an indication that the retention of silica beads is influenced by the rate of flow. At the time of the earlier study a seemingly unrelated observation was made concerning the retention of cell particles, notably red blood cells and yeast cells. The elution pattern of both cell samples indicated diameters which were much larger than those observed microscopically; that is, the cells eluted earlier than expected. For red cells the elution position corresponded to a calculated diameter of about 13 $\mu$m, whereas the largest diameter for this disk-shaped particle is known to be around 7.4 $\mu$m. The yeast cells (diameter around 5.7 $\mu$m)

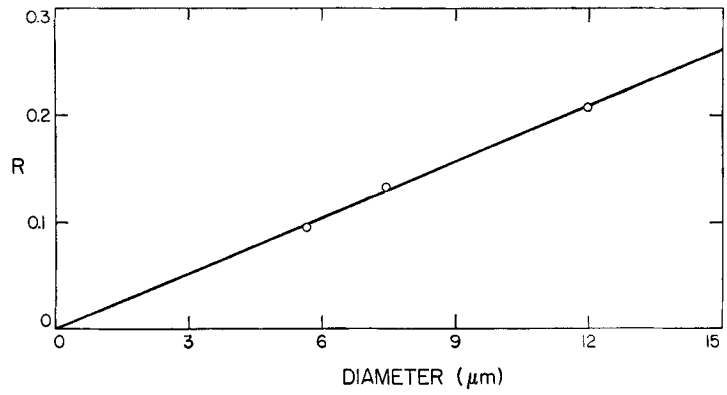


FIG. 2. Retention ratio $R$ (from Fig. 1) of silica beads plotted as a function of bead diameter. The slope of the line, 174.2 $cm^{-1}$, corresponds to $3/w\gamma$. Since the channel thickness $w$ is 0.0127 cm, $\gamma$ in this case calculates to 0.74.



similarly eluted at a position corresponding to a particle of 11.4 $\mu$m diameter.

The physical difference between the silica beads and cell particles is primarily one of density. However, since the biological particles are not strictly spherical, the influence of shape should also not be overlooked. In order to examine further the factors influencing retention in steric FFF, a study was made on the retention of pairs of spherical beads of different densities but of comparable diameters under conditions of different flow velocities.

## THEORY

In conventional FFF an injected solute sample is forced by the field to concentrate as a layer at one of the walls of the flow channel (*5*). The interplay between concentrating and diffusing processes leads to an exponential solute distribution

$$c(x) = c_0 \exp(-x/l) \tag{1}$$

where $c_0$ is the solute concentration at the wall (where $x = 0$) and $l$ is the mean effective layer thickness. Coordinate $x$ varies between 0 and $w$, which interval spans the thickness of the flow channel. As the carrier liquid moves with laminar flow through the channel, zones with large $l$ values will protrude more into the rapid moving flow lines than the compact wall-

hugging zones characterized by small $l$ values. As a result, a large $l$ will lead to a large zonal velocity through the channel. The retention ratio $R$, which equals the ratio of this velocity to the average velocity of the carrier, can be shown to depend exclusively on the ratio $l/w$ in the following manner:

$$R = 6\frac{l}{w}\left[\coth\frac{w}{2l} - 2\frac{l}{w}\right] \tag{2}$$

In the limit of small $l$'s this expression can be approximated by

$$R \simeq 6\frac{l}{w} \tag{3}$$

However, the force acting on the sample particles is in some cases large enough to generate $l$ values which are small in comparison with the radius $a$ of the particle. It is this inequality ($l < a$) which distinguishes steric FFF from the conventional FFF technique.

As stated previously, the retention of a zone dominated by steric FFF effects is primarily governed by the dimensions of the particle, with large particles moving ahead of small ones in the separation channel. The retention ratio has been approximated (*2*) by

$$R \simeq 6\frac{a}{w} + 6\frac{l}{w} \tag{4}$$

This expression corresponds to the movement of the particle with the velocity of the carrier liquid at the $x$ coordinate coinciding with the center of gravity of the particle. With spheres touching the wall, the zone velocity would be that of the carrier at $x = a$. However, the presence of a viscous drag to particle rotation would reduce this velocity and as a result affect the observed retention. Therefore, Eq. (4) may be modified to the form

$$R \simeq 6\gamma\frac{a}{w} \tag{5}$$

where $l/w$ (the term for normal FFF) has been neglected in comparison to $a/w$, and the factor $\gamma$ (less than unity) accounts for the drag-induced reduction in velocity. The slope of the line relating $R$ to particle radius in Fig. 2 indicates that $\gamma$ is around 0.7.

Immediately upon injection of the sample into an FFF separation channel, the flow is stopped and all the particles in the injected zone are allowed to settle or "relax" to the wall region. The time $\tau$ allotted to this procedure is calculated as the time required for a particle in the proximity

of one wall to travel with the field the entire distance $w$ to the opposite wall (*4*):

$$\tau = w/U \tag{6}$$

where $U$ is the field-induced velocity, which in the case of sedimentation FFF is expressed as

$$U = M(\Delta\rho/\rho_s)\cdot G/6\pi\eta a = \frac{2a^2\Delta\rho G}{9} \tag{7}$$

Here $M$ is the particle mass, $G$ the gravitational field strength, $\Delta\rho$ the density difference between particle and solvent, $\rho_s$ the solute (particle) density, $\eta$ the solvent viscosity, and $a$ the particle radius.

## EXPERIMENTAL

The current sizing experiments were carried out using two instruments whose detailed construction has been described elsewhere (*6*). One flow channel is flat and positioned horizontally. This unit is designed to use the earth's gravitational field for the transport of sample particles to the wall region. Its length $L$ and thickness $w$ are 0.86 m and 127 $\mu$m, respectively; the void volume is 1.25 ml.

The second flow channel is designed to spin in a centrifuge. The axis of rotation is positioned horizontally so that particles will not settle toward the channel edge and disturb the movement of the zone within the channel. The channel thickness $w$ is again 127 $\mu$m and length $L$ is 0.80 m. The void volume is 2.05 ml. Spin rates (revolutions per minute) are counted electronically by means of a slotted disk mounted on the shaft and rotating between a light source and a photomultiplier. The rotor radius is 7.7 cm for this unit.

The carrier liquid is pumped through both systems by means of Chromatronix variable speed pumps, and the effluents are monitored by flow-through UV detectors operating with a 254-nm light source. The detector in line with the centrifugal channel is an LDC Model 1205 with a cell volume of 10 $\mu$l; the signal is fed to an Omniscribe flat bed recorder. The horizontal channel is equipped with an Altex Model 135 detector whose cell volume is 8 $\mu$l. This detector is operated in line with a Honeywell "Electronic 19" Chart Recorder.

Retention data are calculated from chart distances of void peak and sample peak as measured from the onset of the flow subsequent to relaxation. The samples used in this study included microporous silica

spheres (density approximately 2.1 g/ml) of narrow size distribution (5.6 ± 0.9 μm) previously characterized by transmission electron microscopy (*4*). These beads were provided by Dr. Jack J. Kirkland of E. I. du Pont de Nemours & Co., Wilmington, Delaware. As a low-density analog (1.05 g/ml), we used polystyrene latex beads from Polyscience, Inc. (Lot No. 2009–2) of approximately the same average diameter: 5.7 ± 1.5 μm.

All runs involving latex beads were carried out using a 0.1% FL70 detergent (Fisher Scientific Co.) dissolved in doubly distilled water. This carrier also contains 0.02% sodium azide as a bacteriocide. The silica beads were first studied in 0.01 *M* ammonia solution which was the carrier used in Ref. *4* as well as in Fig. 1, and were subsequently run in the above detergent solution. The temperature in all cases was 25°C.

## RESULTS

The previously observed (*4*) variation in the retention parameter *R* for silica spheres with changes in fluid velocity was clearly evident beyond experimental error. The deviations from values predicted by Eq. (4) were, however, small, and a suitable operating velocity could be chosen which gave good agreement between particle radii determined experimentally and radii recorded microscopically. Deviations from theory are more substantial for the low-density latex spheres. The earlier-than-expected elution for these samples suggests particle sizes substantially larger than the true values, just as had been observed for the cell particles discussed in the Introduction.

In order to further probe this finding, mixtures of silica and latex beads of essentially the same size were injected into the flow channel using 0.1% detergent as carrier. The presence of detergent was deemed necessary to prevent aggregation of the latices. (Shifting from the original ammonia solution to detergent was found to have no effect on the retention of the silica.) From Fig. 3 it is evident that such mixtures can be readily separated into their components in spite of the prediction of Eq. (4) of almost identical retentions. The figure shows that the silica fraction of bead diameter 5.6 μm is baseline resolved from a polystyrene latex of bead diameter 5.7 μm at a flow of 24 ml/hr.

A more in-depth velocity study of this pair of beads is reported in Fig. 4. From this graph it is evident that for flow rates ranging from 2 to 100 ml/hr there is no flow rate at which the retention of the two samples is identical. However, the difference in *R* is reduced by using a low flow

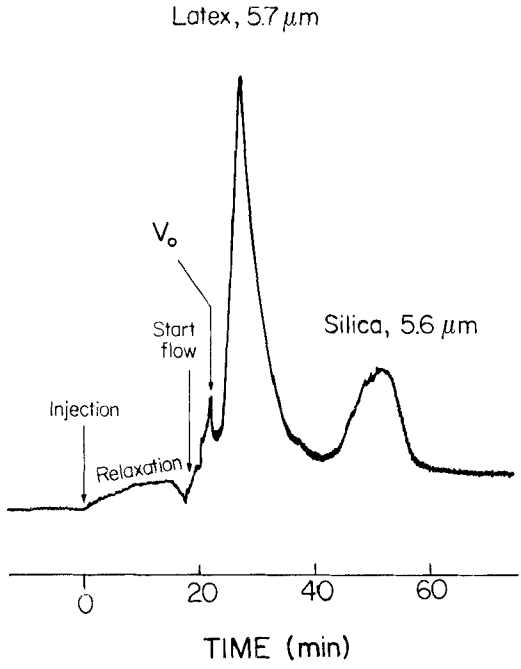


FIG. 3. Separation of a mixture of silica beads of diameter 5.6 $\mu$m and polystyrene latex beads of diameter 5.7 $\mu$m. The carrier liquid is water containing 0.1% FL70 detergent flowing at a rate of 23.5 ml/hr.



velocity. Figure 4 also illustrates the importance of including the "drag factor" $\gamma$ in Eq. (5) to account for the departure from simple theory. The exact magnitude of $\gamma$ has yet to be determined.

The increase in $R$ with higher flow shows that a particle increasingly departs from the velocity experienced by the carrier at the coordinate position $x = a$. This suggests that the particle drifts away from the wall and into faster moving liquid strata as flow rates increase. Velocity-dependent forces are known to act on particles under the influence of shear flow (*7*); these forces may be responsible for such a drift (see below).

The presence of a lift force would manifest itself by an increase in zone velocity provided the magnitude of the force was sufficient to overcome the field-induced force which initially transported the particle to the wall region. The interplay of the forces is illustrated in Fig. 5. This competing-force model would predict that the retention of low-density latex beads is

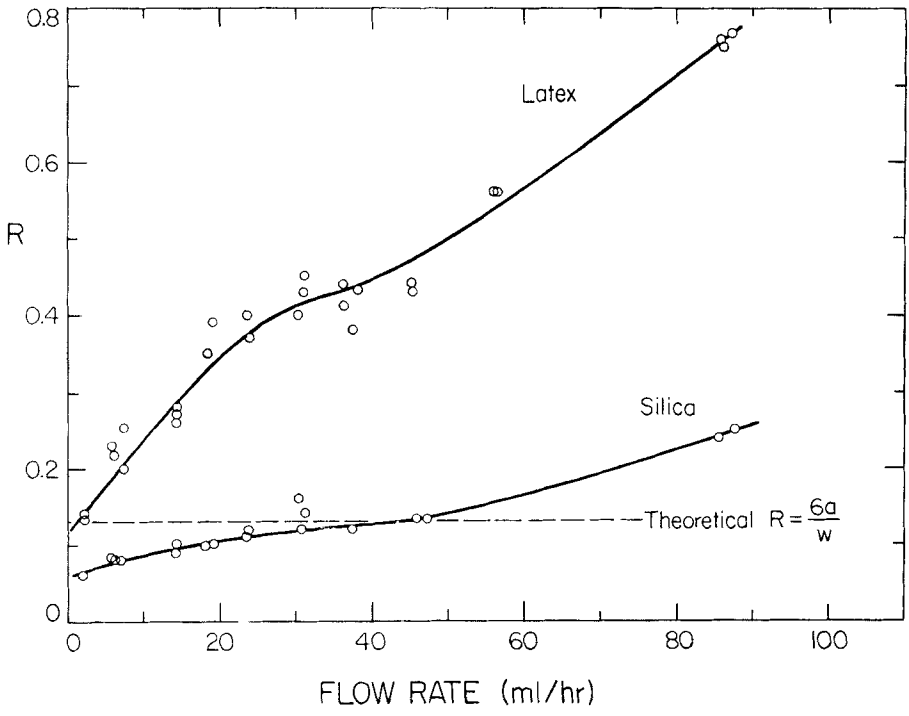


FIG. 4. Retention ratio $R$ plotted as a function of flow rate for 5.6 $\mu$m diameter silica beads and 5.7 $\mu$m diameter latex beads. The theoretical value, $R = 6a/w$, is indicated by the horizontal line.

much more susceptible to change through changes in fluid velocity than is the retention of the high-density silica spheres. A test of this model is to observe the migration process in the presence of a stronger acceleration field than that offered by the earth's gravity. Under such conditions a lift force, even on low-density particles, should be largely offset by the increased sedimentation force, and the particles should be subject to greater retention even at high fluid velocities.

For this test, experiments were carried out in a flow channel which was originally designed to be used for sedimentation FFF (*8*). Figure 6 illustrates the velocity dependence of retention for a sample of latex beads (particle diameter 5.6 $\mu$m) under fields of 1 and 10 gravities. As predicted, for a given velocity the retention observed under the higher field is stronger (lower $R$ value) than retention with the weaker field. A further demonstra-

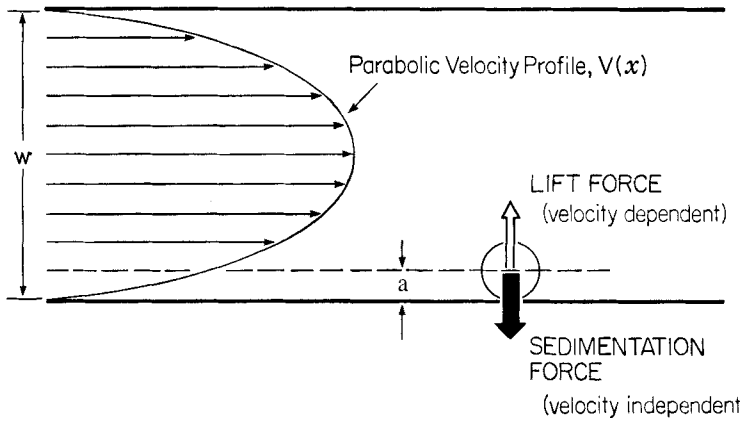


FIG. 5. Schematic illustration of the interplay between the velocity-dependent lift force and the field-induced sedimentation force, the latter being independent of velocity. A net lift force presumably moves the particle into regions of faster flow, thereby leading to an increased $R$ value.



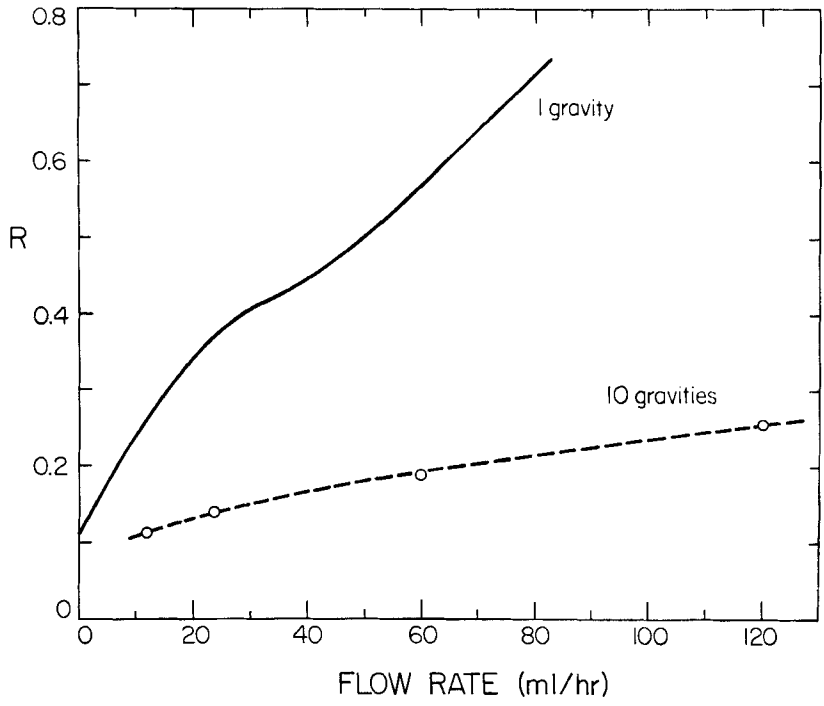


FIG. 6. Retention versus flow rate plots of latex particles of 5.7 $\mu$m diameter at sedimentation fields of 1 and 10 gravities. (The 1 gravity curve is from Fig. 4).

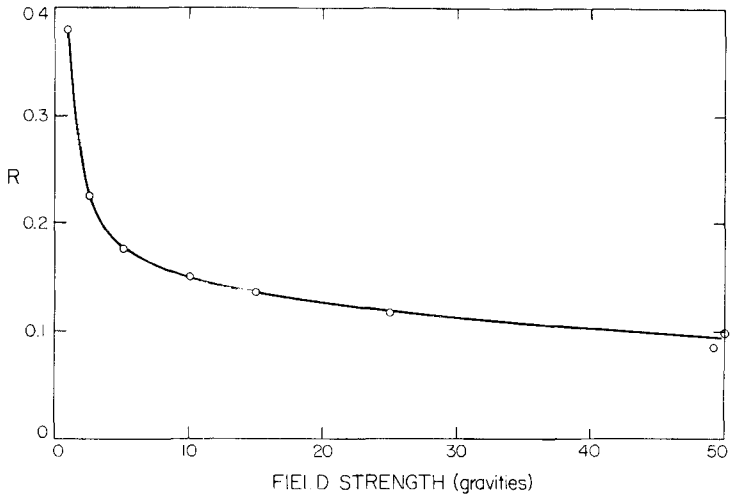


FIG. 7. Retention ratio $R$ of latex particles of 5.7 $\mu$m diameter as a function of sedimentation field (in gravities) at a fixed carrier flow rate of 24 ml/hr.



tion of this effect is provided by Fig. 7 in which retention of the 5.6 $\mu$m diameter latex beads is plotted as a function of field strength at a fixed fluid velocity of 24 ml/hr. The trend toward stronger retention with higher fields is quite evident. A comparison between Figs. 4 and 7 indicates that at the 24 ml/hr flow rate a field strength of approximately 16 gravities is needed to retain 5.7 $\mu$m latex beads to the same degree as is observed for 5.6 $\mu$m silica beads under the influence of 1 gravity.

## DISCUSSION

In an article entitled "The Lift on a Small Sphere in a Slow Shear Flow" (7), P. G. Saffman gives a mathematical expression for the velocity-dependent force which acts on a suspended particle in a direction perpendicular to flow in a tubular duct. According to this treatment, the position-dependent force $F(y)$ depends on the average flow velocity $\langle v \rangle$, particle radius $a$, fluid density $\rho$, and viscosity $\eta$ in the following manner:

$$F(y) = 81.2(\eta \cdot \rho)^{1/2} \cdot a^2/h \cdot (3 \cdot \langle v \rangle \cdot y)^{1/2} \cdot \Delta v \qquad (8)$$

where $y$ is the radial position coordinate ($y = 0$ at the center of the tube), $h$ is the tube radius, and $\Delta v$ is the velocity difference between the particle and fluid at the position of the particle's center of gravity. This treatment

was developed for regions where wall effects can be neglected and is consequently not strictly applicable in the case of steric FFF where particles are touching or nearly touching the wall of the flow channel. It does, however, demonstrate the existence of velocity-dependent lift forces and thereby suggests a qualitative explanation for anomalous retention at high flows.

Our results suggest that the field, which forces the sample particles toward the wall, tends to offset the velocity-induced lift force, thus increasing the retention and resolution of low-density particles in steric FFF systems. Therefore, an increased field permits the use of higher flow velocities with a concomitant increase in separation speed. Such an increase in field strength has the additional advantage of allowing a reduction in relaxation time, providing another incremental increase in analysis speed. For example, under the influence of 1 gravity, relaxation times for low-density latex beads and animal cells are roughly several minutes. Typically, a separation will involve a 10-min relaxation time and another 10 min to complete the run. With increased sedimentation fields, the relaxation times may be cut to 30 sec or less, and since higher fluid velocities can be employed under high-field conditions without distorting the desired retention, separation times of one or a few minutes are a likely prospect. Therefore we are led to believe that steric FFF with a controllable field strength (for example, using a centrifuge rather than the earth's gravity) will have important advantages in speeding the separation process. A controllable field strength will also permit greater versatility: in the sedimentation case one could choose conditions where separation is based mainly on density differences or size differences, or some desired combination of the two.



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