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A comparison of two quality assessment methods for emulsions[☆]

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Abstract

A common method of assessing the quality of emulsions is to evaluate the size distribution of the globules of the internal phase. The primary aim of this work is to compare the sensitivity of this test to an alternative method. The sizes of the globules of two emulsions, an oral emulsion and a total parenteral nutrition (TPN) emulsion, were determined using a light microscope. Globule size analyses were performed upon preparation and during storage of the emulsions. Using a computer program specially developed for this study, the recorded diameters were placed into size groups and the volumes of each of the measured globules was determined. For each size group, the total volume of all the globules within the group and the volume percentage of the oil phase represented by the group were calculated. The volume distribution of the internal phase across the size groups was found to predict emulsion instability better than the globule number distribution and thus is a better determinant of emulsion quality. This technique may have general application in the evaluation of TPN emulsions and other spheres, such as liposomes.

Keywords: Emulsion stability; Globule size analysis; Total parenteral nutrition; Creaming; Breaking

1. Introduction

Emulsions are two-phase systems, consisting of liquid globules dispersed in an immiscible liquid. The phases are separated by an emulgent sheath around the internal phase. These systems are inherently unstable and, ultimately, the phases will separate completely. With regard to stability, the concerns of the pharmaceutical scientist are (a) what can be done to delay separation and (b) if separation is inevitable, how to predict when an emulsion will break. The second concern will be addressed in this paper.

Breaking or cracking refers to the complete separation of phases. A less severe consequence of the tendency of the globules to separate is creaming, wherein the emulgent sheath still surrounds each globule, but the globules are not evenly distributed throughout the external phase. In an oil-in-water emulsion, the oil globules rise and form a more concentrated layer at the surface of the emulsion. While shaking the container will redistribute the internal phase evenly, creaming brings the globules closer together and hence there is a greater opportunity for the coalescence of adjoining globules. As coalescence continues, larger and larger glob-

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ules are formed. The process of globule coalescence and growth leads, ultimately, to the breaking of the emulsion. Therefore, measurement of the sizes of the globules initially and during storage provides an indication of the stability of the system: the faster the globules increase in size, the lower the stability. An assessment of what constitutes a substantial change in the size distribution of emulsion globules may not be as obvious as it would appear.

Globule size can be measured by several methods. Some examples, with the principle involved stated in parentheses, are: Coulter Counter (electrical conductivity) [1-3], HIAC Analyzer (light blockage) [4-6], Malvern Sizer (light diffraction) [7] and microscopy (direct observation) [8]. Microscopy is a common way of measuring the sizes of emulsion globules. The use of this technique has been facilitated by the availability of commercial image analyzers. Whatever the method of size analysis, it is usual to place the globules into size groups, based on their diameters, and to determine the number of globules in each group. A typical example of results thus obtained is shown in Table 1. The presence of only a few larger-sized globules is often considered evidence of stability or, rather, a lack of instability. It is the contention of the authors that it is not the number of larger-sized globules that is significant, but rather the percentage of the oil phase occupied by these globules.

To assist in explaining this concept, the components of an emulsion (oil, emulgent and water) are depicted in Fig. 1. The oil phase may, in turn, be divided into the volume made up of small globules, the volume made up of medium-sized globules and the volume made up of large globules. For simplicity, the oil phase has been divided into only three size groups in this diagram. To assess the stability of an emulsion, the percentage of the oil phase appearing as large globules should be determined. Considering the example given in Table 1, what volume percentage of the oil phase does the globule in the largest size group constitute? The answer may be surprising.

Table 1
Results of a typical size analysis

Size group (μm)	Number
0-5	10
5-10	22
10-15	12
15-20	3
20-25	2
25-30	1

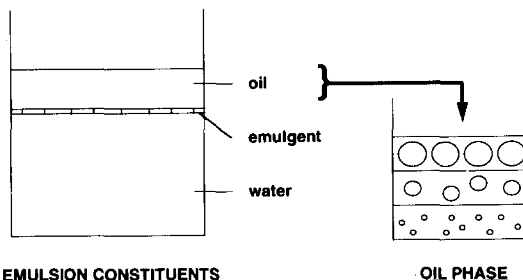

Fig. 1. Diagrammatic representation of emulsion constituents.

2. Computer program

During the initial studies, in order to estimate the volume percentage occupied by globules of a particular size group, each globule within a size group was represented as having a diameter equal to the midpoint of the size group. In this approximation, the total volume of all the globules within a group was the volume of the "average" globule multiplied by the number of globules within the group. For example, suppose one of the size groups was 10-20 μm. If there were 10 globules in this group, it was assumed that each globule had a 15 μm diameter. The volume of this sphere was calculated and multiplied by 10 to obtain the volume of all the globules within this size group.

To test the validity of this approximation, a size analysis was performed, and the volumes of the individual globules within each size group were calculated and summed to obtain

Table 2
Formulae for liquid paraffin emulsion (500 ml)

Ingredient	B.P.	Prepared
Liquid paraffin	250 ml	250 ml
Vanillin	250 mg	-
Chlorform	1.25 ml	1.25 ml
Benzoic acid soln. B.P.[a]	10 ml	10 ml
Methylcellulose 20	10 g	-
Methylcellulose 100	-	5 g
Saccharin sodium	25 mg	-
Water	to 500 ml	to 500 ml

[a] Benzoic acid, 5 g; propylene gylcol, 75 ml; water, to 100 ml.

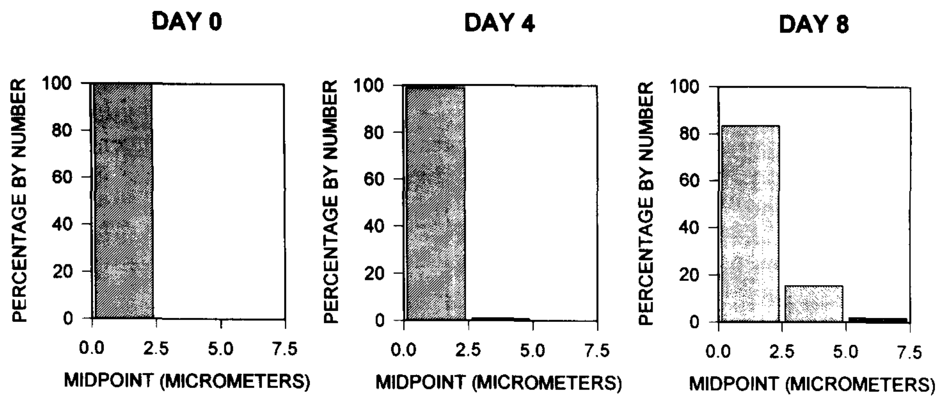

Fig. 2. Size distribution by number for the TPN emulsion.

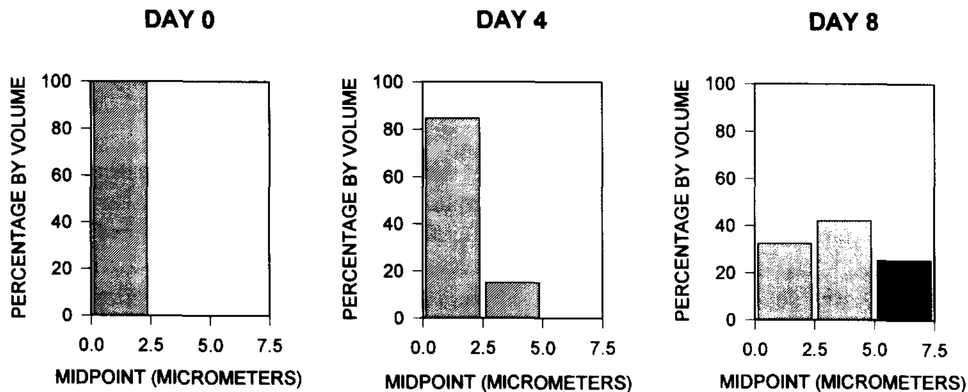

Fig. 3. Size distribution by volume for the TPN emulsion.

the total volume for that group. This value was compared with the estimated value, obtained as described above. The process was repeated for all the groups. It was found that a large error resulted from approximating the globule diameters with the midpoint of the size group. Hence, it is necessary to calculate the volume of every globule from a size determination. To avoid the tedious calculations involved, a computer program was written in Turbo Pascal®.

The program places the globules into different size groups based on their diameters. It calculates the volume of every globule and also the total volume of all the globules within each group (group volume). In addition, it combines the group volumes to obtain the total volume of all the globules measured (total volume). The percentage of the oil phase represented by one particular size group is the group volume divided by the total volume and then multiplied by 100%.

3. Experimental

3.1. Preparation of emulsions

Two emulsions, a liquid paraffin (LP) emulsion and a total parenteral nutrition (TPN) emulsion, were prepared.

The LP emulsion was prepared according to the formula given in Table 2. This emulsion is similar to the LP emulsion described in the British Pharmacopoeia (B.P.), the formula for which is also given for comparison. The preparation of the emulsion is straightforward. In the final step, 250 ml of liquid paraffin was added to 250 ml of the aqueous phase and stirred at 2000 rpm for 5 min, using an IKA RW 20 stirrer (Janke & Kunkel, Germany). The emulsion was not homogenized as required by the B.P. It was packed into test tubes, each containing approximately 12.5 ml.

For the preparation of the TPN emulsion, 10 ml of the standard intravenous (IV) mix-

ture used at Truman Medical Center, Kansas City, MO, USA, was mixed with 10 ml of 10% Intralipid® and 10 ml of 9 mM $CaCl_2$ solution by vortexing for 20 s. The standard IV mixture contained dextrose, amino acids, electrolytes, trace minerals and vitamins. Twenty microliters of heparin (50 000 IU ml^{-1}) was added and the mixture was vortexed for 20 s. The emulsion was packed into test tubes, each containing approximately 1.5 ml.

3.2. Globule size measurement

The emulsions were examined microscopically (Olympus microscope fitted with a 40 × objective lens and a 20 × eyepiece) for the sizes of the globules upon manufacture, and thereafter every week for the LP emulsion and every 4 days for the TPN emulsion.

On the day of the examination, the test tube was inverted five times to distribute the creamed globules. A 30 μl aliquot was removed from approximately the center of the volume of the emulsion in the test tube. This was added to 970 μl of deionized water and the diluted emulsion was gently shaken by inverting five times. Five microliters of the diluted emulsion was removed from the center of its volume and placed on a glass slide onto which a cover slip was positioned. The globules were observed at 800 × magnification and the diameters of 200 globules, from different locations on the slide, were measured using an eyepiece graticule. The graticule was calibrated using a stage micrometer (Nikon, Japan).

The data were processed using the computer program developed for these calculations. The results were plotted as the number of globules per size group versus the midpoint of the size group, as well as the percentage of the oil phase occupied by each size group versus the midpoint of the size group.

4. Results

Fig. 2 is the globule size distribution by number for the TPN emulsion. Upon production of the emulsion, all globules were smaller than 2.5 μm in diameter. On day 4, the diameter of the majority of the globules was still less than 2.5 μm, with some globules appearing in the second size group (2.5–5.0 μm). A significant number of globules were in the second size group by day 8, with some globules in the 5.0–7.5 μm size range.

Globules of diameter larger than 6 μm in the general circulation can cause hypotension, acidosis and emboli in the lungs [1]. Since there were few globules in the 5.0–7.5 μm size range, one might be tempted to consider this emulsion as marginally acceptable.

Fig. 3 is the globule size distribution by volume. On the day of preparation, 100% of the oil phase was made up of globules of diameter less than 2.5 μm. On day 4, a substantial volume percentage of the oil phase was in the second size group (2.5–5.0 μm). This was not observed when the number distribution was considered. On day 8, the globules were

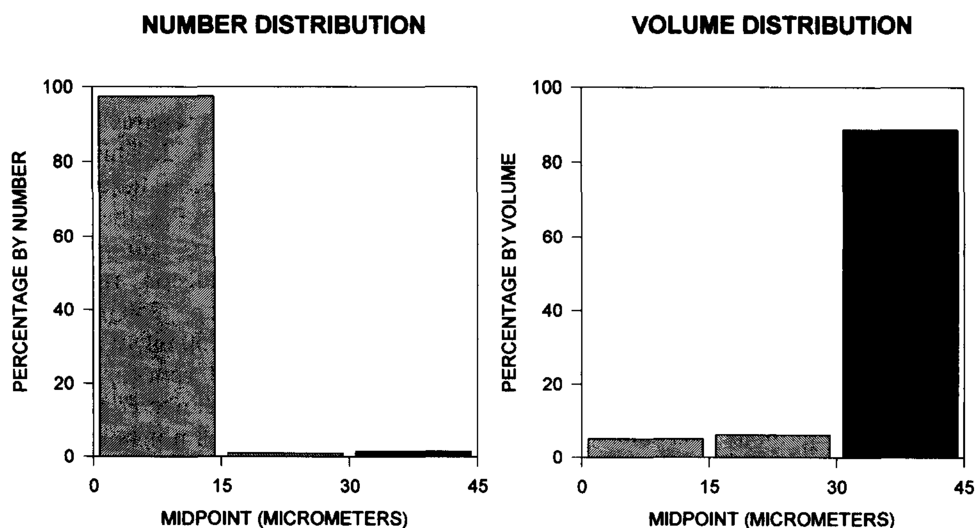

Fig. 4. Size distribution on day 12 for the TPN emulsion.

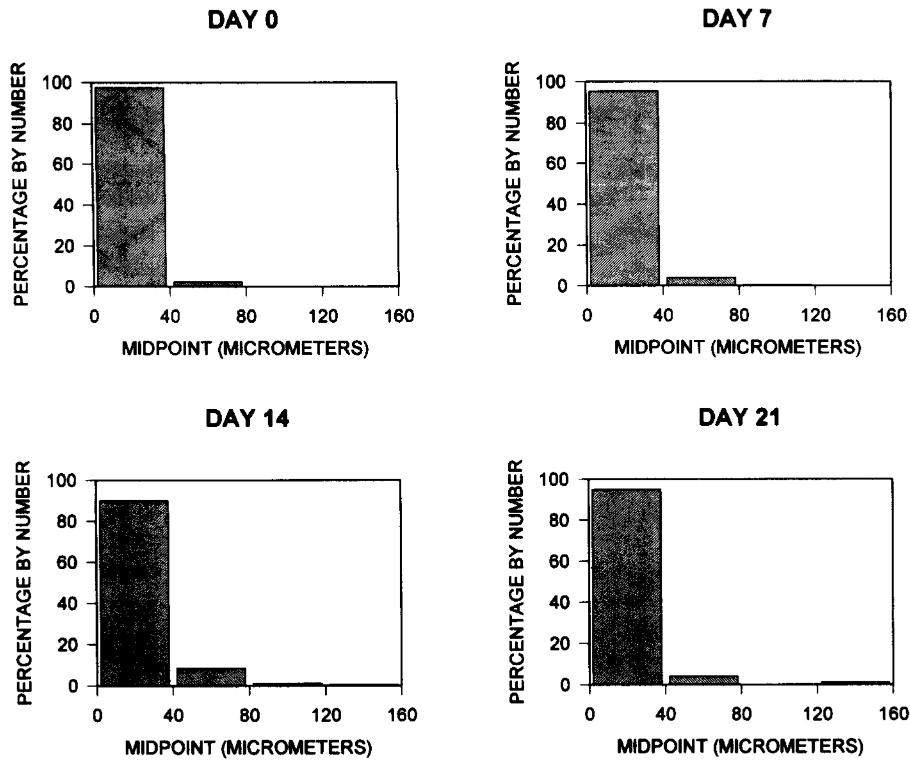

Fig. 5. Size distribution by number for the LP emulsion.

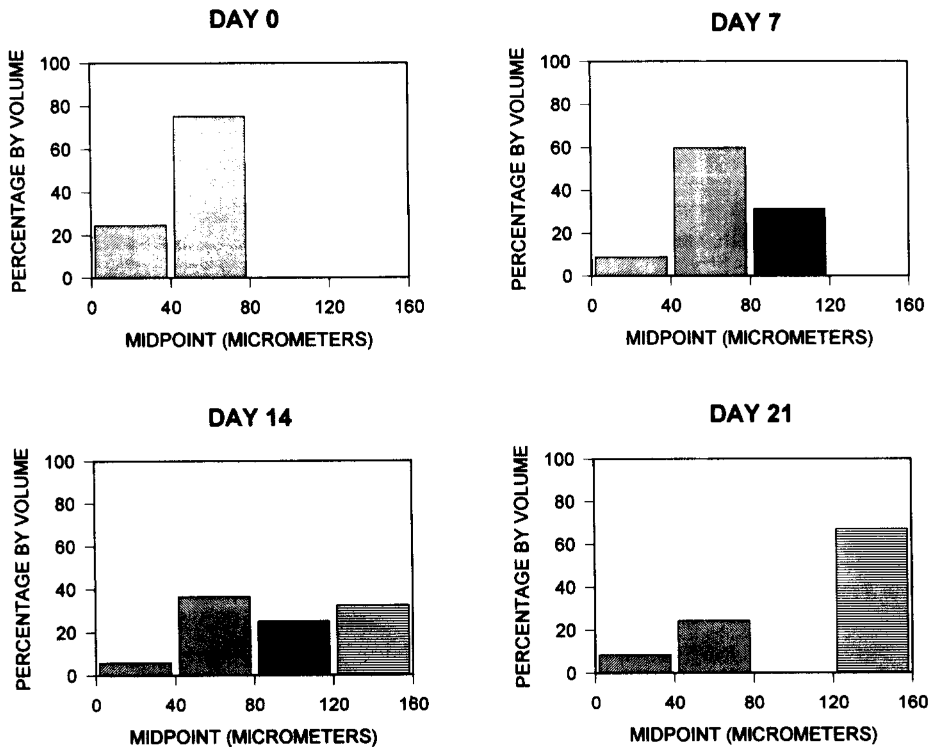

Fig. 6. Size distribution by volume for the LP emulsion.

more or less evenly distributed over the three size groups. With approximately 25% of the oil phase consisting of globules in the 5.0–7.5 μm size range, there is no doubt that this emulsion should not be administered to a patient.

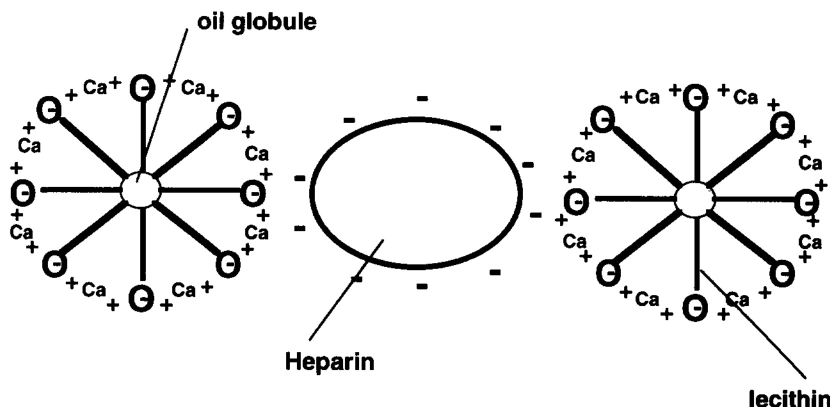

Fig. 7. Interaction between heparin and calcium-stabilized oil droplets (adapted from Ref. [11]).

Fig. 4 compares the number distribution with the volume distribution for the TPN emulsion on day 12. Considering only the stability of the emulsion and not its suitability for injection, one would conclude from the number distribution that the emulsion is still stable. Almost 100% of the globules were less than 15 μm in diameter. The volume distribution presents a totally different picture. Almost 90% of the oil phase was made up of globules in the largest size group (30–45μm), thus indicating that the emulsion was considerably less stable than revealed by the number distribution. This emulsion broke on day 20.

Fig. 5 is the size distribution by number for the LP emulsion. There was hardly any change in the size distribution of the globules over the 21 days of the study, indicating a stable emulsion. Fig. 6 is the size distribution by volume for the LP emulsion. Even on the day of manufacture, the largest percentage of the oil phase was in the second size group (40–80 μm), not in the 0–40 μm size group as for the number distribution. By day 7, a substantial volume of the oil phase was in the third size group (80–120 μm) and, by day 14, in the fourth size group (120–160 μm). By day 21, the major portion of the volume of the globules appeared in the fourth size group. The volume distribution method revealed that the emulsion had changed considerably over the period of the study. This was in sharp contrast to the results from the number distribution analysis. This emulsion broke after approximately 4 months.

5. Discussion

During the initial studies, globule size was measured without using a coverslip. It was observed that the globule shape became distorted and that coalescence occurred as the slide dried. Therefore, a coverslip was used in subsequent experiments.

Because a manual (microscopic) method was used, only 200 globules were measured at each observation. Also, attention was focussed on the differences between the two methods and no formal attempt was made to determine the error involved in the measurements. In subsequent work, an automated procedure will be used to measure a larger number of globules and an analysis of error will be performed.

The unhomogenized LP emulsion and the TPN emulsion are inherently unstable systems. The larger globule sizes and the wide range of sizes in the LP emulsion make it unstable. The presence of the larger initial sizes means that very large, unstable globules can be formed fairly quickly owing to coalescence. The wide size distribution allows closer packing of the globules since small globules fit into the spaces between large globules. Hence, there is a greater opportunity for coalescence.

TPN emulsions are finely balanced mixtures containing soybean oil emulsified with lecithin. Lecithin is a mixture of phospholipids. While the major component, phosphatidylcholine, is neutral at physiological pH, minor components, such as phosphatidylserine, carry a negative charge [7]. Other constituents of the emulsion can affect the delicate balance, especially if they carry an electrical charge. Heparin has been reported to destabilize TPN emulsions [9,10]. This apparently occurs only in the presence of divalent cations, such as calcium ions. The postulated mechanism is depicted in Fig. 7. Lecithin is associated with the oil droplet as shown. If there are divalent cations (such as calcium ions) present in the mixture, they be-

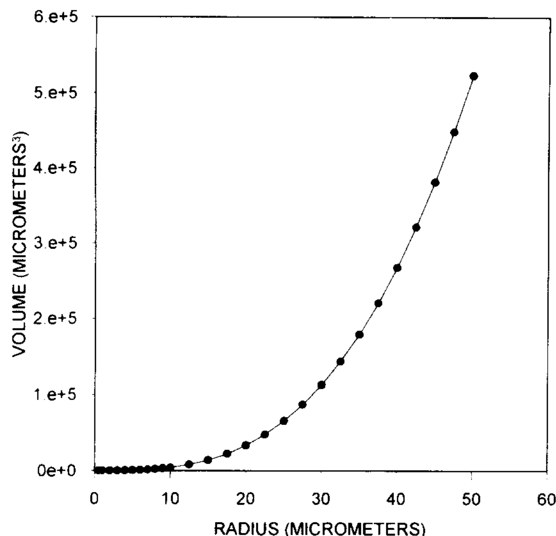

Fig. 8. Relationship between radius and volume of a sphere.

come associated with the lecithin-stabilized globule as indicated. This association further stabilizes the emulsion as the oil globule–lecithin–Ca^{2+} complex repels other similar complexes. However, if heparin, an anionic polyelectrolyte, is present it forms a bridge between adjacent complexes. This causes flocculation, leading to coalescence and, ultimately, to breaking of the emulsion. These unstable systems were chosen since they change relatively rapidly and, hence, the two quality control measures could be compared within a short time.

The reason for the vastly different results obtained by the two techniques can be understood if the volume relationship between small and large globules is considered. Approximately one million globules of 1 μm diameter have the same volume as a single 100 μm globule. In the number distribution technique, globules of either size are given equal weight in quality assessments.

The differences in the results obtained by the two techniques can be further explained by observing the relationship between the radius and the volume of a sphere (Fig. 8). For small numerical values, as the radius is increased, the volume of the sphere increases very slowly. The slope of the initial part of the curve is not steep. For larger numerical values, increasing the radius increases the volume of the sphere dramatically as seen by the very steep slope of the latter part of the curve. This is because the radius (r) is cubed when calculating the volume (V) of the sphere.

$$V = \frac{4}{3}\pi r^3$$

6. Conclusions

The results show that the volume distribution of the internal phase technique is a far more sensitive measure of changes occurring in the emulsion than the number distribution technique. Our perception of small changes in the emulsion is accentuated by the volume distribution technique, whereas these changes are not readily observed with the number distribution method. The volume distribution technique is a better determinant of emulsion quality and can demonstrate the unsuitability of an emulsion at an earlier stage during stability studies. This technique may have utility in the evaluation of TPN emulsions in clinical situations. It may also be useful in the evaluation of other spheres, such as liposomes.

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