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The Release of Macromolecules from Fatty Acid Matrices: Complete Factorial Study of Factors Affecting Release

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Abstract—A replicated complete factorial design to study the main effects and interactions of four factors: bovine serum albumin (BSA) particle size (Factor A); stearic acid particle size (Factor B); BSA loading (Factor C); and compression force (Factor D), on the release of BSA from compressed stearic acid pellets was performed in isotonic phosphate buffer pH 7·4 at 37°C. Samples were withdrawn over 64 h. Analysis of variance of the percentage released at 64 h showed that A, B, and C, but not D, affected the release and the interactions AB, BC, ABC were highly significant. At low loading (5%), the surface release depended on BSA particle size. The release increased when BSA particle size was large. At high loading (20%), more release was shown when stearic acid particle size was large. More release with increasing BSA particle size occurred only when stearic acid particle size was small. It is proposed that release is due to the interconnected pore networks created, not only by BSA particles, but also by the void space between stearic acid particles. These void spaces vary according to particle size-dependent arrangements of stearic acid and BSA particles. An increase in the pellet thickness was observed probably due to the relaxation of compacted stearic acid particles.

Advances in biotechnology have made available a wide range of macromolecular therapeutic agents. Their low bioavailabilities and short half-lives in plasma make the conventional oral and parenteral routes less useful (Siegel & Langer 1984), but polymeric matrix systems are potentially important as implants for delivery of polypeptides and proteins (Siegel & Langer 1990). However, the low cost, low toxicity, and ease of fabrication using lipids suggest that fatty acids could be useful matrix substances (Wang 1987a). Macromolecule-lipid matrices prepared by direct compression showed a sustained release of macromolecules both in-vitro and in-vivo (Wang 1987b; Khan et al 1991).

The mechanism of release of macromolecules from compressed matrices is not well defined; however, some parallels might exist with release from polymeric monolithic systems. The release of macromolecules from the polymeric monolithic system occurs through an interconnected pore network, which is created by solid drug particles initially present in the matrix (Siegel et al 1989). The pores are randomly distributed within the matrix and communicate through narrow throats (Siegel & Langer 1990). The formulation parameters, such as drug particle size and drug loading, influence release (Bawa et al 1985). Generally, an increase in drug particle size or drug loading causes an increase in release rate (Siegel et al 1989). Interconnected pore networks could be formed in compressed matrices; therefore, similar effects of drug particle size and drug loading on the release could be expected for compressed matrices.

Release from compressed delivery systems is also affected by formulation parameters such as particle size of the matrix substance and the compression force. Packing arrangements of drug particles and matrix substance particles depends on the shape and size of the particles (Martin et al 1983), and this should affect pore characteristics. Compression force also affects the pore structure and size. Bodmeier & Chen (1989) showed the effect of compressibility on the internal void volume.

For a predictable controlled release of macromolecules from a compressed matrix, more understanding of the effects of drug particle size, matrix substance particle size, drug loading and compression force is required. Factorial-design experiments have been shown to be an efficient way of analysing the complex interactions involved in a complete tablet formulation and indicate the significant factors for detailed study (Sanderson et al 1984). This paper reports a replicated complete factorial design to study these effects on the release of bovine serum albumin from compressed stearic acid matrices.

Materials and Methods

The materials used were bovine serum albumin (BSA) (A-7030, Sigma, USA) and stearic acid (S-4751, Sigma, USA). Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride were analytical grade (BDH, UK) and sodium azide was chemical grade (Hopkin & Williams Ltd, UK).

Factorial design of the formulations

A complete factorial design for four factors at two levels resulted in $2^4 = 16$ trials. The design was replicated by preparing duplicate batches of each treatment combination. The factors and levels are shown in Table 1 and the composition of the formulations in Table 2.

Preparation of BSA pellets

BSA and stearic acid powder were sieved using standard sieves (Endecotts Ltd, UK). These powders were stored in a desiccator over silica gel at 6°C. Duplicate batches (500 mg)

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Table 1. Factors and levels investigated.

Code	Factors	Low level	High level
A	BSA particle size (μm)	63–125	250–500
B	Stearic acid particle size (μm)	63–125	250–500
C	Loading (%)	5	20
D	Compression force (ton)	2	3·5

of each combination were prepared by geometrically mixing the BSA and stearic acid in 2 × 10 cm modified glass test-tubes in which three baffles (12 mm long and a depth of 3 mm) were fitted at the bottom. Mixing was achieved using a vortex mixer (Chiltern, USA) at speed 4 for 30 s after each addition of stearic acid and for 1 min after the last addition. The mixture was compressed with flat stainless steel punches (5 mm) using a hydraulic press (Fred S. Carver Inc., USA). The required compression force was reached within 10 s and maintained for 2 min. Mean weight of pellets was 30·35 mg with standard deviation of 0·31 mg. Thickness of pellets was also measured using a micrometer (Moore & Wright Ltd, UK). The pellets were stored in a desiccator over silica gel at 6°C until used.

Release studies

The weight and thickness of each pellet were measured before and after the study. The medium used for the release study was isotonic phosphate buffer pH 7·4 (PBS) with 0·1% sodium azide and was equilibrated to 37°C for at least 1 h before use. Duplicate pellets from each combination were each placed in a 30-mL glass test-tube with 3 mL medium. The test-tubes were capped with Teflon-lined caps and then left unshaken in a 37°C water bath. Samples (0·5 mL) were drawn at 1, 2, 4, 8, 16, 32, and 64 h and 0·5 mL PBS was replaced at each sampling time. The samples after appropriate dilution with PBS were directly injected into the HPLC system. At the end of the experiment pellets were removed, washed in PBS for a few seconds and dried in a vacuum desiccator over silica gel at room temperature for 48 h. Weight and thickness were measured and the pellets were analysed for BSA content as described below. Another three pellets from each formula were also assayed for BSA content to test batch content.

Table 2. Composition of the formulations.

	Factor level in experimental units			
Trial	A	B	C	D
1	1	0	0	0
2	1	1	0	0
3	1	1	1	0
4	1	1	1	1
5	0	1	1	1
6	1	0	1	1
7	0	1	0	1
8	1	0	1	0
9	1	1	0	1
10	0	1	1	0
11	0	0	1	1
12	1	0	0	1
13	0	1	0	0
14	0	0	1	0
15	0	0	0	1
16	0	0	0	0

0, low level; 1, high level.

HPLC analysis method for BSA

The BSA content of the samples and pellets was assayed using high-performance size-exclusion chromatography (HPSEC). A Jasco system (Japan) comprising pump (Model 851-PU), autosampler (Model 851-AS) and UV detector (Model 875-UV) set at 220 nm was used. The column was a Protein-Pak 300 SW (10 μm, 7·8 × 300 mm, Waters, USA) with a Protein-Pak 125 guard column (37–53 μm, 2·1 × 30 mm, Alltech, USA). A column (5 × 150 mm) packed with silica gel (Code No. 15049, BDH, UK) was connected between pump and autosampler to protect the analytical column packing from dissolution of packing material. Mobile phase was 10 mM phosphate buffer pH 6·5 containing 150 mM NaCl using a flow rate of 1·0 mL min^{-1}. Samples (100 μL) were injected into the system. Peak heights were measured by the Delta chromatography system (Version 4.05, Digital Solutions Pty Ltd, Australia).

Standard curves were prepared using BSA solutions of 40, 100, 200, 300, 400 and 500 μg mL^{-1}. For analysis of BSA content in the pellets, the same concentration of standard BSA solutions were prepared and were kept in an ice bath for at least 30 min. Three millilitres of each cold standard solution was transferred to a glass homogenizer. Blank stearic acid pellets were ground with these solutions for 30 s in an ice bath. Solutions were filtered (0·45 μm, HAWP 01300, Millipore, USA), the first half of filtrate discarded and the remaining filtrate used for analysis of BSA content.

The calibration curves in PBS pH 7·4 were linear and the within-day and between-day coefficients of variation were less than 5%. Calibration curves of standard solutions in the presence of stearic acid pellets were linear and showed lower slope and intercept. The calibration curves were constructed each day.

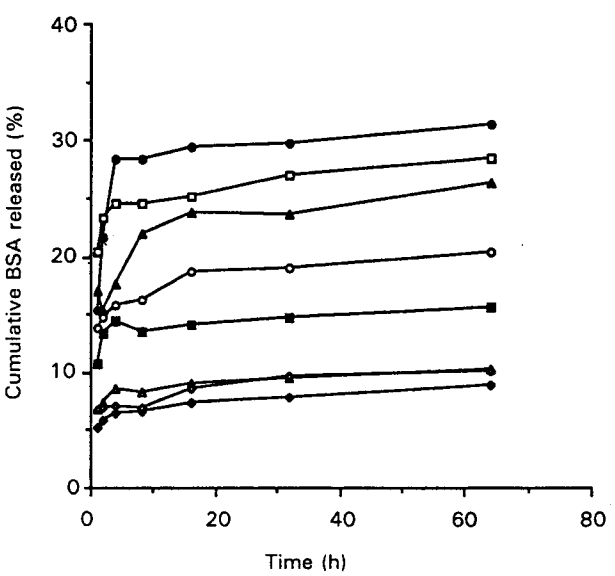

FIG. 1. Release of BSA from pellets containing a 5% loading of BSA and prepared using high and low level particle sizes and compression forces. Points are the means of duplicate pellets. Symbols are trial 1 (●), 2 (○), 7 (■), 9 (□), 12 (▲), 13 (△), 15 (◆), 16 (◇).

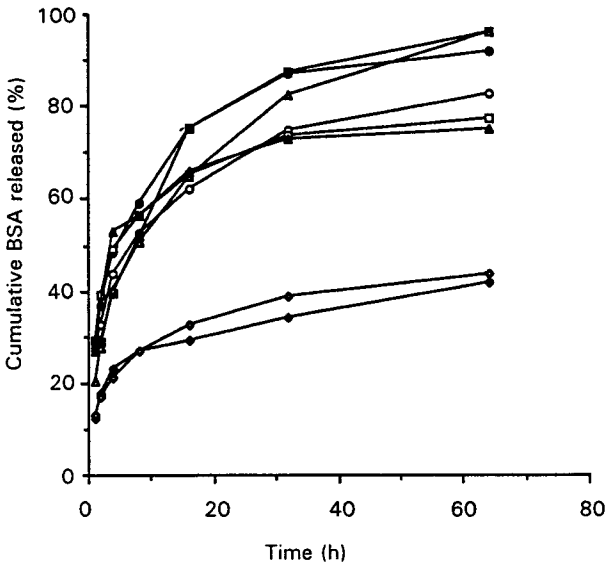

FIG. 2. Release of BSA from pellets containing a 20% loading of BSA and prepared using high and low level particle sizes and compression forces. Points are the means of duplicate pellets. Symbols are trial 3 (●), 4 (○), 5 (■), 6 (□), 8 (▲), 10 (△), 11 (◆), 14 (◇).

Data analysis

Cumulative percentages released (P) at each time were calculated as:

$$P = \frac{AM_t \times 100}{AM_{64} + R_{64}} \quad (1)$$

where AM_t is the cumulative mass released at time t, AM_{64} and R_{64} are the cumulative mass released and the residual BSA in the pellet, respectively, at 64 h. The data were subjected to analysis of variance.

Results

The BSA content of pellets calculated by the sum of the cumulative amount released at the last time point and the amount remaining in the pellet after the experiment, showed agreement with the contents as determined from analyses of three pellets from each batch.

Cumulative percentage release of BSA over 64 h for 5% and 20% loadings are shown in Figs 1 and 2, respectively. In the case of 5% loading, the burst effect was followed by only a slight increase in the release. Cumulative percentages released at 64 h for 5% loading varied between 8 and 34% (Fig. 1). The release profiles of 20% loading showed a burst effect followed by a continuous release (Fig. 2). Release varied from 38 to 97% at 64 h.

Analysis of variance of cumulative percentages released at 64 h showed significant effects for BSA particle size (A), stearic acid particle size (B), and BSA loading (C) ($P < 0{\cdot}001$), while compression force was not significant (Table 3). The interactions of A with B, B with C, and A with B with C were also significant ($P < 0{\cdot}001$). These interactions are shown in Fig. 3.

A correlation ($r = 0{\cdot}820$) was found between increase in thickness of pellets and cumulative percentages released at 64 h (Fig. 4).

Discussion

The system under study consists of compressed matrix stearic acid (matrix substance) and BSA (active ingredient) particles. This system is different from monolithic matrices in which the active drug particles are suspended in the homogeneous matrix substance.

Siegel et al (1989) suggested that release from a monolithic matrix occurs through a porous network of tortuous channels created by the drug particles. At high loading, the drug forms a complete interconnected pore network and release is due to dissolution and diffusion through this network. Increased loading provides simpler pathways (low tortuosity) and greater porosity for diffusion (Rhine et al 1980). At low loading, incomplete interconnected pore networks are formed; therefore release is incomplete although a burst effect is observed due to release of particles at the surface of the matrix. Burns et al (1990) claimed that the percolation threshold, the loading when the pore network starts to be complete, is 33·8% loading.

In compressed matrices, drug particles may also touch each other forming an interconnected network similar to a monolithic system. Furthermore, compressed matrices have

Table 3. Analysis of variance based on cumulative percentages released at 64 h.

Source of variation	df	Mean squares	F	P
BSA particle size (A)	1	1546·2	47·40	<0·001
Stearic acid particle size (B)	1	2024·1	62·05	<0·001
BSA loading (C)	1	25784·9	790·50	<0·001
Compression force (D)	1	0·3	0·01	0·927
A*B	1	1253·5	38·43	<0·001
A*C	1	19·4	0·60	0·451
A*D	1	4·9	0·15	0·704
B*C	1	2149·1	65·88	<0·001
B*D	1	14·6	0·45	0·513
C*D	1	34·4	1·05	0·320
A*B*C	1	587·4	18·01	0·001
A*B*D	1	6·0	0·18	0·674
A*C*D	1	2·3	0·07	0·793
B*C*D	1	105·2	3·23	0·091
A*B*C*D	1	48·9	1·50	0·238
Error	16	32·6		
Total	31			

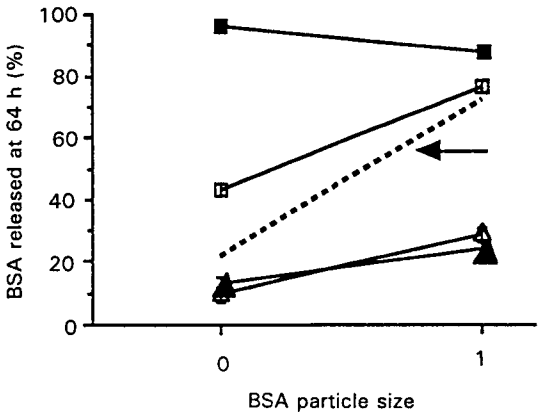

FIG. 3. Interaction between BSA particle size, stearic acid particle size and loading. BSA particle size: 0, 63–125 μm; 1, 250–500 μm. △, Stearic acid 63–125 μm at 5% loading. ▲, Stearic acid 250–500 μm at 5% loading. □, Stearic acid 63–125 μm at 20% loading. ■, Stearic acid 250–500 μm at 20% loading. - - - - - Expected surface release. Error bars show the pooled estimate of the standard error.

void spaces between matrix particles which could, in part, affect the release.

Our results show that for 5% loading only a slight increase in the release occurred after the burst effect and the cumulative percentages released at 64 h were low. This is consistent with most BSA particles being trapped in the matrix and only the particles at or near the surface dissolving into the medium. At the higher loading of 20% the continuous release after the burst effect and the high cumulative percentages released at 64 h suggest that dissolution and diffusion of non-surface BSA particles through the interconnected pore networks are occurring. Thus loading had a highly significant effect on release.

The variation of the release at 64 h (8–34% for 5% loading and 38–97% for 20% loading) relates to the differences in BSA and stearic acid particle sizes. This indicates that the pore networks were created not only by interconnecting BSA particles but also by the void between stearic acid particles. The void spaces in the pellets would vary according to the size-dependent arrangements of stearic acid and BSA particles.

It might be expected that the compression force would affect drug release from matrix pellets prepared by direct compression, since it will affect the porosity of the pellets. Indeed, this has been reported previously (Carli et al 1981; Bodmeier & Chen 1989). However, analysis of variance shows that the compression force range of 2–3·5 ton did not have a significant effect on release in this study.

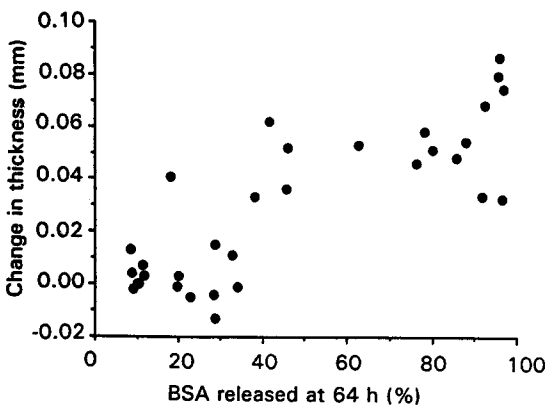

FIG. 4. Scattergram of changing thickness of pellets and cumulative percentage release at 64 h. The correlation coefficient is 0·820.

As proposed above, at low loading, only surface release occurred. The expected surface release from a cylindrical pellet where release occurs from both ends and the sides of the cylinder can be calculated by:

$$F = \frac{\pi r^2 h - \pi(r - d_{vn})^2(h - 2d_{vn})}{\pi r^2 h} \quad (2)$$

where F = fraction of BSA in contact with the surface (within one BSA particle diameter of surface), r = pellet radius, h = pellet thickness (height) and d_{vn} = average volume-number diameter of BSA particles. The particle size distribution of BSA was determined by microscopy in which the sizes of 500 particles were measured. The average volume-number diameter of BSA particles was 115·9 and 457·8 μm for sieve size 63–125 and 250–500 μm, respectively. The fractions of BSA in contact with the surface calculated from equation 2 are 0·23 and 0·74 for BSA size 63–125 μm and 250–500 μm, respectively. Thus, fraction surface release is dependent on BSA particle size. This is shown diagrammatically in Fig. 5.

At low loading, release increased as BSA particle size increased as predicted, but there was no significant difference between small and large stearic acid particle size (Fig. 3). However, the release was lower than the expected surface release. This phenomenon cannot be explained simply. Most waxes are soft and should flow around the drug particles during compression (Schwartz et al 1968). Benita et al (1984) also discussed this effect in relation to the interaction between drug and ethylcellulose/stearic acid matrices on release. In addition, it has been studied for compressed tablets made using one moving plunger. Particles on the upper side (moving plunger side) were moved more than those on the opposite side and this gave rise to the higher density on the upper side of the compact (Van Groenou 1981). Thus, lower release than expected is possibly because of coverage of BSA particles by melted stearic acid or differences in BSA particle rearrangements between upper side and lower side during compression, leading to the diminished release on one side.

At 20% loading, release was greater than the expected surface release. This confirms that apart from surface release, BSA is released from the inside of the pellet as the interconnected pore network is formed. Under these conditions the effect of the stearic acid particle size is complex, as shown by the significant three component interaction (Table 3). When stearic acid particle size is large the release is higher than that for the smaller size. Furthermore, at small stearic acid particle size, release increases with increase in BSA particle size, whereas when stearic acid particle size is large, release decreases when BSA particle size increases (Fig. 3). This can be explained by the effect of particle size on void space. Pore size of the void space between larger particles of stearic acid is larger than between smaller particles. More non-surface release could thus occur due

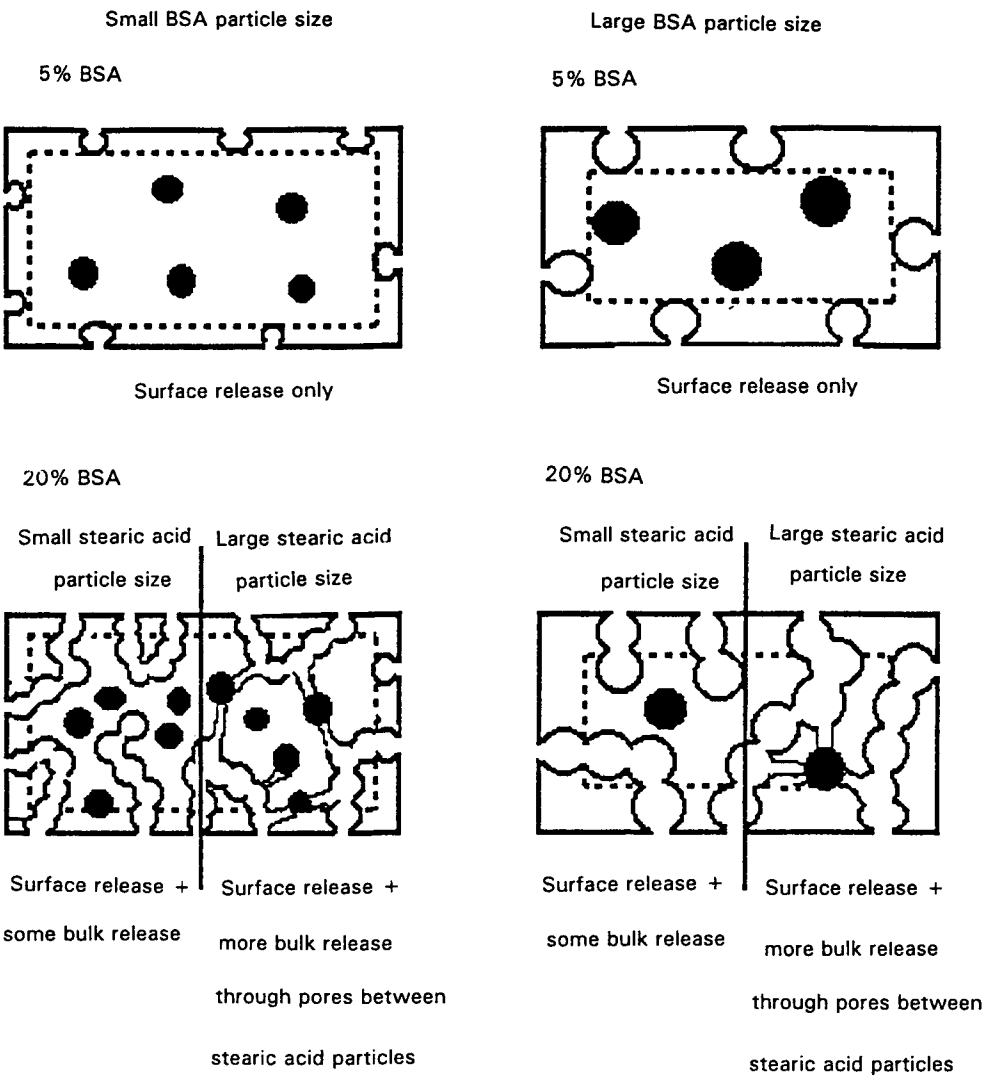

FIG. 5. Proposed model of release from compressed matrices showing effects of BSA and stearic acid particle sizes and loading. Black regions represent BSA particles, holes represent dissolved BSA particles. More bulk release occurs due to pores between stearic acid particles, especially when stearic acid particles are large.

to release not only through the BSA pore network, but also through the pore network between stearic acid particles (Fig. 5).

Apart from the influence of stearic acid particle size, BSA particle size also affects the release. When stearic acid particle size was small, more BSA release occurred when BSA particle size was larger, suggesting that the release occurred mainly from the surface and the inside connected particles with little or no release through small-size pores between small stearic acid particles. On the other hand, for large stearic acid particle size, the release decreased when BSA particle size was large (Fig. 3). When BSA particle size is small the number of particles and the effective surface area are large. These small BSA particles could spread around the surface of stearic acid particles. This would give a greater chance for interconnecting BSA channels to be formed and thus more release. When BSA particle size becomes larger a decreased effective surface area could result in a smaller chance of the BSA particles interconnecting with each other; the release then decreases.

There was an increase in thickness of the pellets which is significantly correlated with BSA release (Fig. 4). Blank pellets did not show a significant increase in either PBS or 0·05% BSA in PBS. Therefore, increase in thickness is probably due to the relaxation of compacted stearic acid particles. As BSA dissolves and diffuses out, media probably penetrates between the particles leading to a loosening of the stearic acid particles and an increase in thickness.

We conclude that at low BSA loading, release is predominantly from the surface layer of the pellet and the thickness of this layer increases with BSA particle size. At high BSA loading, apart from surface release, BSA diffuses between stearic acid particles in addition to release via interconnecting BSA channels.

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