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Report

Manufacture and Properties of Erythromycin Beads Containing Neutron-Activated Erbium-171

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To evaluate the effects of a neutron activation radiolabeling technique on an enteric-coated multiparticulate formulation of erythromycin, test quantities were produced under industrial pilot scale conditions. The pellets contained the stable isotope erbium oxide (Er-170), which was later converted by neutron activation into the short-lived gamma ray-emitting radionuclide, erbium-171. *In vitro* studies indicated that the dissolution profile, acid resistance, and enteric-coated surface of the pellets were minimally affected by the irradiation procedure. Antimicrobial potency was also unaffected, as determined by microbiological assay. Neutron activation thus appears to simplify the radiolabeling of complex pharmaceutical dosage forms for *in vivo* study by external gamma scintigraphy.

KEY WORDS: neutron activation; erbium-171; erythromycin; drug delivery system; gamma scintigraphy; radiolabeled dosage forms; scanning electron micrography; erythromycin dissolution; erythromycin antimicrobial activity.

INTRODUCTION

The use of external scintigraphy to evaluate the *in vivo* behavior of radiolabeled dosage forms has gained considerable popularity since its initial application in our laboratories (1). To date, the technique has been applied to the study of tablets (2), capsules (3), suppositories (4), and enema preparations (5).

In early gamma scintigraphy studies, a radioactive isotope (technetium-99m or indium-111) was incorporated into study formulations. The radiolabeled dosage form was then administered via its appropriate route, followed by external scintigraphy and image processing. The use of this labeling technique has a number of limitations: dosage forms had to be prepared under small-batch conditions rather than under large-scale conditions replicating commercial production methods; also, to avoid radioactive contamination of industrial facilities and equipment, the dosage form usually had to be prepared at the imaging facility. Thus, it is not feasible to use active isotopes to radiolabel dosage forms which require lengthy manufacturing procedures or highly specialized industrial equipment for their preparation.

To overcome these limitations, Parr *et al.* (6,7), introduced a technique that incorporates trace amounts of a stable isotope (barium-138 sulfate, erbium-170 oxide, or samarium-152 oxide) into the bulk formulation along with other excipients. Routine manufacturing procedures are applied and the prepared dosage form is then subjected to a neutron flux which converts the stable isotope into a radioactive gamma ray-emitting isotope (^{139}Ba, ^{171}Er, ^{153}Sm) that can easily be followed *in vivo* by external gamma scintigraphy.

The physical properties of certain stable isotopes are well suited to the neutron activation method of radiolabeling. As shown in Table I, these isotopes, when activated, have half-lives that range from 63 min to 47 hr, permitting sufficiently long scan periods for definitive scintigraphic observations, with minimal radiation exposure. These isotopes also emit gamma rays in the optimum imaging range of most commercially available gamma scintillation cameras (100 to 300 KeV).

As shown in Table I, these isotopes have high-neutron capture cross sections and are readily activated by neutron bombardment. In contrast, many of the excipients used in pharmaceutical preparations (carbon, hydrogen, nitrogen, phosphorous, and some halogens) have low-neutron capture cross sections and are not readily activated by irradiation. Thus, by neutron activation of selected stable isotopes, it is possible to label a dosage form with a marker of high radionuclidic purity, using industrial pilot-scale methods and equipment similar to that employed commercially.

Two stable elements found in many pharmaceutical preparations (sodium and potassium) are easily converted to their radioactive isotopes (sodium-24 and potassium-42) by neutron activation. In products containing sodium or potassium salts of an active drug, these radionuclidic contaminants can constitute the majority of the radioactivity pro-

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Table I. Nuclear and Physical Characteristics of Stable Isotopes Used in the Neutron Activation of Radiolabeled Dosage Forms

Isotope		Neutron capture cross section (barms)	Half-life (hr)	Photon emitted (keV)	Water solubility (mg/100 ml)	Radiation exposure per 50 μCi (rads)
Stable	Radioactive					
Ba-138	Ba-139	0.4	1.38	169	0.22	0.0016
Er-170	Er-171	5.6	7.52	112,124 296,308	0.49	0.0065
Sm-152	Sm-153	210.0	46.7	103	0.054	0.0080

duced by neutron activation. Problems associated with these isotopes can be reduced or eliminated by carefully specifying irradiation conditions or by selective use of an isotope with appropriate physical properties.

For example, in products containing sodium or potassium salt, Sm-152 is selected and irradiation time is increased. The resulting Sm-153 has a comparatively long half-life and will remain present in amounts sufficient for scintigraphy after sodium-24 and potassium-42 have decayed to negligible levels of gamma emission. The radionuclidic purity of pharmaceutical dosage forms radiolabeled by this procedure regularly exceeds 95% and, in some instances, may be greater than 99%.

The safety of these rare earth isotopes has been studied extensively in animals (8,9). When administered under physiological conditions, these isotopes form insoluble hydroxides and phosphates that are not readily absorbed (8). The oral safety of these isotopes is also supported by gastrointestinal transit studies in both animals and man (10).

The neutron activation technique affords versatility and safety in radiolabeling intact dosage forms; further, it permits the use of different isotopes with differing physical properties (Table I) for maximum flexibility in labeling dosage forms that require complex manufacturing procedures.

In the present study, an enteric-coated multiparticulate formulation containing erythromycin and the stable isotope, erbium oxide, was manufactured under industrial pilot scale conditions and subsequently radiolabeled using the neutron activation technique. *In vitro* tests were conducted to study the effects of neutron activation on the integrity of the above dosage form and its erythromycin content.

MATERIALS AND METHODS

Pellet Preparation. Erythromycin base (200 g), croscarmellose sodium, and erbium oxide (2.67 g; 96.89% erbium-170) were dry-blended in a planetary mixer (Hobart) for 5 min. The blend was then granulated with an aqueous media containing a binder; if needed, additional water was incorporated to produce a mass suitable for extrusion. The mass was extruded through a single screw extruder (Brabender) and spheronized in a marumizer (Elanco). The pellets were dried overnight at 40°C, then screened, and the 12–18 mesh fraction was collected for further processing.

Preparation of the Coating Formulation. Kaolin was dispersed in water to produce a suspension. An antifoam emulsion and triethyl citrate were added to the suspension while stirring. Finally, a water-based enteric coating material was added to the mixture and stirred for another 10 min prior to coating the pellets (see Table II).

Analysis of Erbium Oxide Content. The content of stable erbium oxide in the erythromycin pellet formulation was determined by placing known amounts of pellets in a neutron flux of 4.1×10^{13} n cm^{-2} sec^{-1} for 2 min. After irradiation, the activity in each sample was determined using a high-resolution germanium–lithium [Ge(Li)] detector; the amount of erbium-171 produced was calculated by comparing the activity of each sample against a known standard. The radionuclidic purity of irradiated pellets was also determined using a germanium–lithium detector (Teledyne Isotopes) interfaced to a multichannel analyzer (Packard).

Dissolution Tests. Dissolution tests were performed in a USP Apparatus I (rotating baskets) on a total of 12 aliquot samples (420 mg each) of the enteric-coated erythromycin pellets containing erbium oxide. Of these samples, six were nonirradiated pellets and six had been irradiated for 55 sec in a neutron flux of 4.1×10^{13} n cm^{-2} sec^{-1}. Each sample was placed in a 40-mesh USP dissolution basket, rotated at 50 rpm in 900 ml of a 0.06 *N* hydrochloric acid for 60 min, and subsequently suspended in 900 ml of a 0.2 *M*, pH 6.8, phosphate buffer at 37°C. At 30 and 60 min 3 ml of dissolution medium was removed, filtered through a 0.45-μm filter, and analyzed by high-pressure liquid chromatography (HPLC) to determine their erythromycin content.

Acid Resistance Test. The acid resistance of the enteric-coated erythromycin pellets was tested before and after irradiation. Samples of the erythromycin-containing pellets weighing about 420 mg were placed in a 40-mesh USP dissolution basket and were suspended in 900 ml of 0.06 *N* hydrochloric acid solution maintained at 37°C. The basket was rotated at 50 rpm for 3 hr; the beads were then removed, rinsed, and dissolved in methanol. Samples (20 μl) were withdrawn from the methanolic solution and analyzed by HPLC to determine erythromycin content.

HPLC Analysis of Erythromycin. Analysis for erythromycin content was performed by injecting 20 μl of each sample onto an Altex Ultrasphere 5-μm ODS column and monitoring the absorbance at 215 nm. A mobile phase consisting of 1.6 g of potassium dihydrogen phosphate, 450 ml of acetonitrile, 550 ml of water, and 0.2 ml of *N,N-*

Table II. Coating Conditions

Flow rate	1–2 ml/min
Liquid nozzle	1.1-mm diameter
Nozzle position	Bottom spray
Fan capacity	10–12
Atomization air	0.2–0.4 bar
Air inlet temperature	45–50°C

dimethyloctylamine was used with a flow rate of 0.8 ml/min. Under these chromatographic conditions, erythromycin eluted at 2 min.

Determination of Irradiation Effect on Antimicrobial Activity. The antimicrobial activity of the erythromycin was determined using a Kirby-Bauer susceptibility test (11). Samples of irradiated and nonirradiated beads weighing about 420 mg were dissolved in 100 ml of methanol; a 10-μl sample from each solution was placed on a sterile disk. The methanol was evaporated and the disks were placed on an agar plate that had been inoculated with *S. aureus*. The agar plate was incubated at 37°C for 24 hr and antimicrobial activity was determined by comparing zones of inhibition.

Scanning Electron Micrography (SEM). To observe any physical changes in the surface of the enteric coating, SEMs were obtained using an ISI DS130 scanning electron microscope with a beam voltage of 9 keV. To prepare samples for SEM, randomly selected pellets were applied to a wet carbon-painted surface and dried. A 25-nm gold–palladium coating was then applied and scanning electron micrography was performed.

Statistical Analysis. A two-sample comparison test ($P < 0.05$) was used to compare the acid resistance, erythromycin content, dissolution profile, and antimicrobial activity of the pellet formulation before and after irradiation.

RESULTS AND DISCUSSION

The effect of gamma irradiation on drugs (12), polymers (13), and various chemical compounds (14) has been evaluated in several studies. However, few investigations have examined the effects of neutron irradiation on the physicochemical properties and *in vitro* behavior of pharmaceutical dosage forms.

Recently, Parr and Jay (15) have shown that the incorporation of stable isotopes, such as erbium and samarium oxides, at low concentrations (<1%, w/w, of the dosage form) does not affect the hardness, disintegration characteristics, or dissolution profile of the various solid dosage forms. These studies have also shown that irradiation times of up to 15 min had no effect on hardness. However, a linear relationship between irradiation time and tablet disintegration time has been observed, when irradiation times of 2 min or more are used. The same authors also used this technique to radiolabel an ibuprofen-containing slow-release table (16) and established that the procedure had no effect on the active drug moiety of the formulation.

Table IV. Percentage Recovery of Erythromycin from Irradiated[a] and Nonirradiated Pellets After Exposure to Acid Test

Sample No.	Percentage acid resistance: Nonirradiated pellets	Percentage acid resistance: Irradiated pellets
1	94	92
2	96	89
3	103	98
Mean ± SD	98 ± 5	93 ± 5

[a] Pellets were irradiated in a neutron flux of 4.1×10^{13} n cm^{-2} sec^{-1} for 55 sec.

The present study was undertaken to examine the effects of neutron activation on the stability of an acid-sensitive drug such as erythromycin in an enteric-coated multiparticulate dosage form. The ability to radiolabel this preparation without affecting the formulation may permit the direct evaluation of commercially available multiparticulate dosage forms of erythromycin *in vivo*.

Prior to *in vitro* studies, the erbium oxide content of the erythromycin pellets was determined, using neutron activation techniques. Results of a 2-min irradiation indicated that the pellets contained 0.0067 mg of erbium oxide per mg of pellet, with a radionuclidic purity greater than 95% erbium-171.

The USP dissolution test for erythromycin was performed as previously explained. Table III shows the percentage of erythromycin released at 30 and 60 min from the irradiated and nonirradiated enteric-coated pellets in a 0.2 *M* phosphate buffer solution (pH 6.8). The data show that the irradiation procedure significantly ($P < 0.05$) decreased the amount of erythromycin released at 30 min but did not have a significant effect on the total amount released at 60 min: the total amounts released for the nonirradiated and irradiated beads were 91.9 and 94.0%, respectively. Presently, the cause for this decrease in drug release at 30 min is being evaluated. These results meet the USP specification that not less than 80% of the labeled amount of $C_{37}H_{67}NO_{13}$ has dissolved within 120 min.

Table III. Dissolution of Irradiated and Nonirradiated Erythromycin Pellets in 0.2 *M* Phosphate Buffer (pH 6.8): Percentage of Erythromycin[a] Dissolved in 60 min

Sample No.	30 min: Nonirradiated pellets	30 min: Irradiated pellets[b]	60 min: Nonirradiated pellets	60 min: Irradiated pellets[b]
1	79.2	60.5	105.5	90.9
2	74.2	62.0	90.7	90.0
3	75.2	67.5	83.9	93.2
4	75.2	68.1	83.2	96.6
5	70.9	63.0	95.0	94.8
6	70.6	67.0	93.2	98.5
Mean ± SD	74.2 ± 3.2	64.7 ± 3.2	91.9 ± 8.2	94.0 ± 3.3

[a] Erythromycin determinations were performed by HPLC.

[b] Pellets were irradiated in a neutron flux of 4.1×10^{13} n cm^{-2} sec^{-1} for 55 sec.

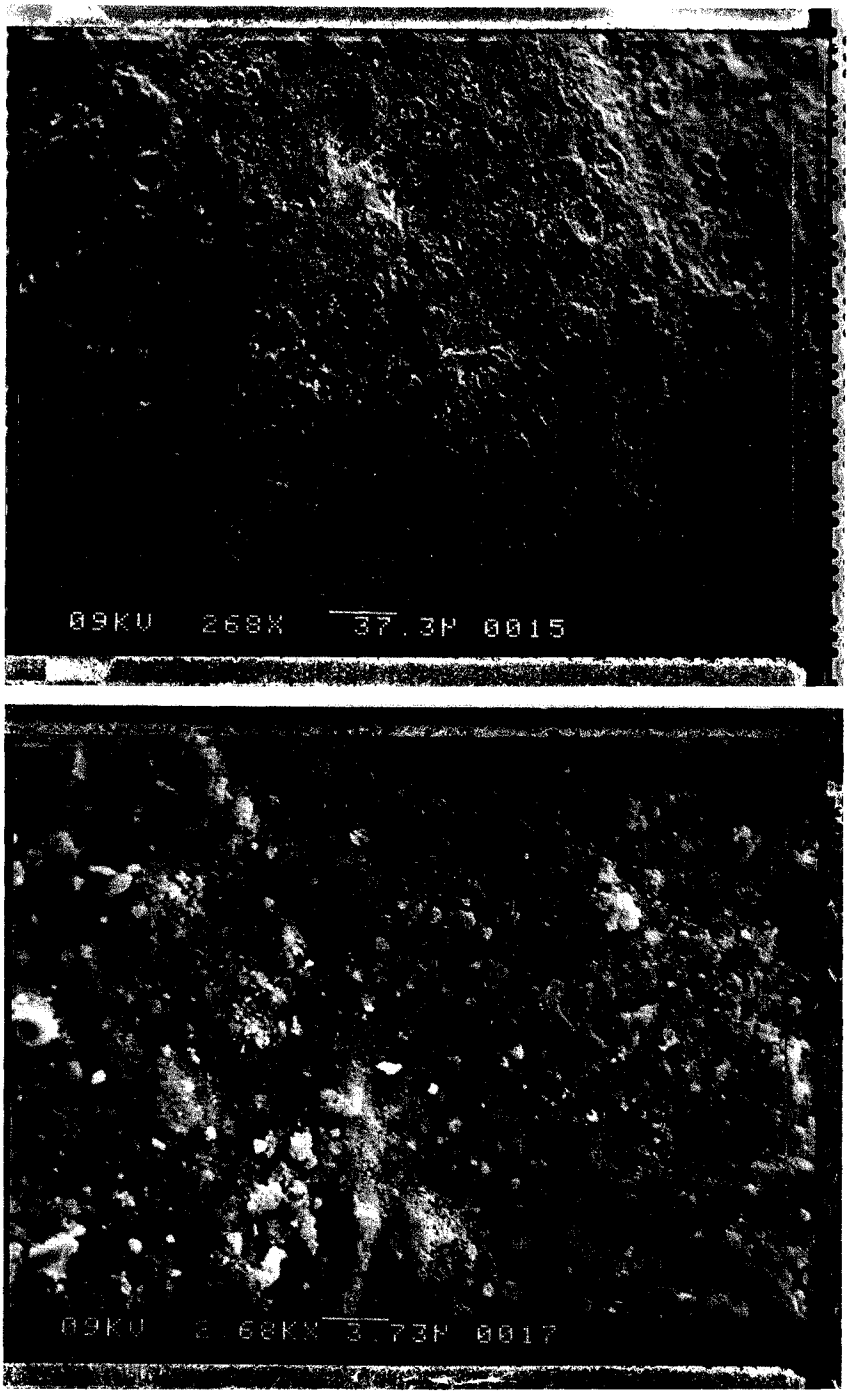

Fig. 1. SEMs of the enteric-coated pellets containing the erythromycin and erbium before being irradiated for 55 sec in a neutron flux of 4.1×10^{13} n cm^{-2} sec^{-1} (a) Bar = 37.3 μm; (b) bar = 3.73 μm.

Since erythromycin is extremely acid-labile, it is important to determine whether the irradiation procedure affects the acid-resistant coating of the preparation. A significant decrease in acid resistance could lead to decreased *in vivo* bioavailability and, therefore, to decreased serum concentrations. The data shown in Table IV indicate that, although there is an observable decrease in acid resistance of the pellets, the decrease was not statistically significant ($P < 0.05$) and is within acceptable limits for such an enteric-coated formulation.

To determine the potential cause of this decrease in acid resistance, SEMs of the pellets were obtained before and after irradiation (Figs. 1 and 2, respectively). A comparison of these SEMs at low magnification shows that the coating of the nonirradiated pellets appears to be more continuous than that of the irradiated pellets. At higher magnification, signs

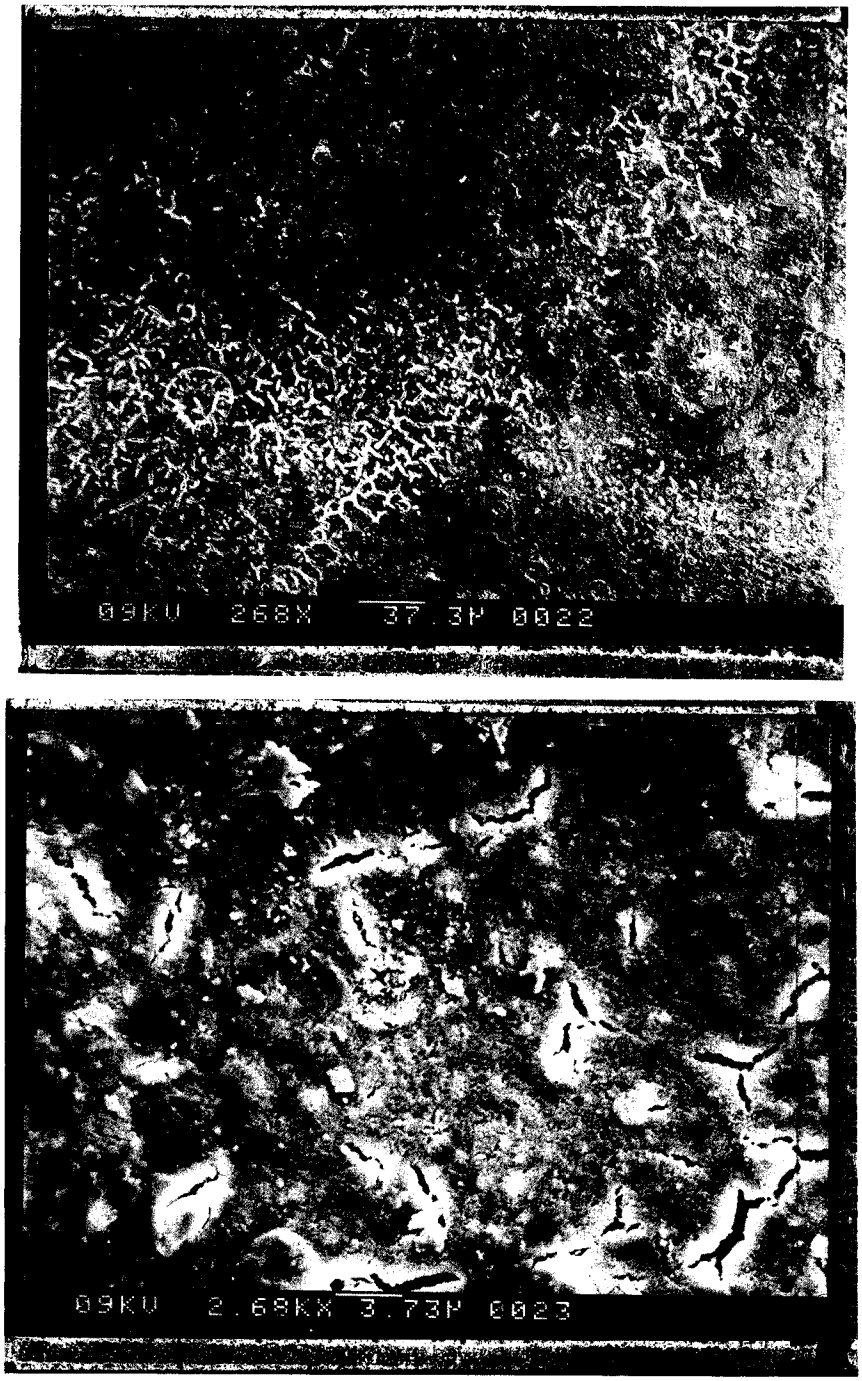

Fig. 2. SEMs of the enteric-coated pellets containing erythromycin and erbium after being irradiated for 55 sec in a neutron flux of 4.1×10^{13} n cm^{-2} sec^{-1}. (a) Bar = 37.3 μm; (b) bar = 3.73 μm.

of cracks in the coating material were observed in both irradiated and nonirradiated pellets; however, the irradiated pellets appear to be more porous, possibly explaining slight changes in their acid resistance. The data indicate that minor changes in the enteric coating did not significantly alter the integrity of the preparation.

HPLC data revealed no change in the erythromycin content of the pellets after irradiation. A microbiological assay was also performed on the pellets to ensure that the erythromycin retained its *in vitro* antimicrobial activity. (The *in vivo* bioavailability of erythromycin from various dosage forms is also determined by microbiological assay.) The results of a modified Kirby-Bauer antimicrobial assay (11) are shown in Table V. The irradiation procedure did not appear to have a significant effect ($P < 0.05$) on the antimicrobial activity of erythromycin contained in the pellet formulation.

Table V. Effect of Neutron Irradiation on the Antimicrobial Activity of Erythromycin-Containing Pellets

Sample No.	Zones of inhibition (mm)	
	12-hr incubation (mm)	24-hr incubation (mm)
Nonirradiated		
1	26	25
2	26	26
3	27	26
4	27	26
Mean ± SD	26.5 ± 0.6	25.8 ± 0.5
Irradiated[a]		
5	27	26
6	26.5	26
7	26	25
8	25	25
Mean ± SD	26.3 ± 0.9	25.5 ± 0.6

[a] Pellets were irradiated in a neutron flux of 4.1×10^{13} n cm^{-2} sec^{-1} for 55 sec.

In contrast, detrimental effects have been reported with ionizing radiation used to sterilize ampicillin (17), neomycin (12), and antipseudomonal penicillins (18). This finding is an important indication that the neutron activation radiolabeling method can be used to radiolabel erythromycin products and thereby permit the noninvasive evaluation of these products *in vivo*.

CONCLUSIONS

The results obtained from the present study indicate that the introduction of a stable isotope into an enteric-coated erythromycin pellet formulation, and its subsequent neutron activation to a gamma-emitting radionuclide had little or no effect on the dosage form and did not appear to affect the antimicrobial properties of the erythromycin contained in this pellet formulation.

The findings demonstrate the feasibility of neutron activation as a method for activating stable dosage forms produced under industrial pilot scale conditions. This approach is of particular importance to investigators wishing to observe through noninvasive techniques the *in vivo* behavior of dosage forms that must be prepared by lengthy and/or complex industrial processes. Such studies may shed light on factors that influence the wide variability observed in erythromycin bioavailability from patient to patient and among different dosage formulations.

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