Dual-Isotope Imaging of Neutron-Activated Erbium-171 and Samarium-153 and the *in Vivo* Evaluation of a Dual-Labeled Bilayer Tablet by Gamma Scintigraphy

George A. Digenis,[1,2] Erik P. Sandefer,[1] Robert M. Beihn,[3] and Alan F. Parr[4]

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INTRODUCTION

Since the initial applications of gamma scintigraphy (1) and dual-isotope imaging (2) for the evaluation of pharmaceutical dosage forms, the significance of correlating drug plasma level with *in vivo* transit behavior of a delivery device has become widely recognized (3–12). This technique is considered noninvasive and safe to use in humans. However, the primary disadvantage of the procedure has been the restrictions dictated by the use of short lived radioisotopes. Conventional radiolabeling techniques typically use indium-111 ($t_{1/2}$ = 2.7 days, γ = 172, 247 keV) or technetium-99m ($t_{1/2}$ = 6.0 hr, γ = 140 keV), both of which require the dosage form to be manufactured at the imaging facility. Consequently, *in vivo* scintigraphic evaluation has been limited to simple dosage forms.

Recent work has shown that such difficulties can be circumvented by neutron activation of a stable isotope present at milligram quantities within the formulation (4,11–18). By this technique, routine manufacturing procedures can be employed and, therefore, permit the manufacture of dosage forms under industrial scale conditions. Radiolabeling is then completed by exposing the dosage form to a neutron flux, and the stable isotope is converted by an (n,γ) reaction to a radioactive isotope which can be evaluated *in vivo* by gamma scintigraphy. Pertinent theoretical discussions of neutron activation as applied to the radiolabeling of pharmaceutical dosage forms have previously been reported (13–16).

In vitro studies were undertaken to determine the ability to identify and simultaneously monitor two neutron activated isotopes, samarium-153 ($t_{1/2}$ = 46.7 hr, γ = 103 keV) and erbium-171 ($t_{1/2}$ = 7.52 hr, γ = 308, 296, and 112 keV), when present as two separate dosage forms. Additionally, to further demonstrate the versatility of the neutron activation technique, an *in vivo* study was performed whereby a bilayer tablet was dual labeled with both ^{171}Er and ^{153}Sm. It is proposed that concomitant imaging of neutron activated ^{171}Er- and ^{153}Sm-labeled delivery systems will be valuable for direct *in vivo* comparisons of complex dosage forms whose manufacture is limited to special industrial procedures.

MATERIALS AND METHODS

Chemicals

The lanthanides used for neutron activation were enriched erbium oxide (96.8% ^{170}Er) purchased from Oak Ridge National Laboratories (Oak Ridge, TN) and samarium oxide purchased from Alfa Products (Danvers, MA). Other tablet excipients included hydroxypropylmethylcellulose (Klucel HF, Aqualon Co., Wilmington, DE), anhydrous lactose USP, magnesium stearate USP, and dicalcium phosphate USP.

Sample Preparation

Single-Labeled Tablets for in Vitro *Studies*

Nondisintegrating single-labeled erbium-171 and samarium-153 samples used for *in vitro* phantom counting and image resolution experiments were radiolabeled by neutron activation. The ^{171}Er-radiolabeled samples were prepared from a mixture of dicalcium phosphate, erbium oxide, and magnesium stearate (88.75:1.25:10.00, w/w). A 400-mg tablet (9.5-mm diameter) was compressed at 5000 lb of pressure via a Carver press. The tablet containing ^{170}Er was subsequently irradiated for 75 sec (flux = 8×10^{13} neutrons cm^{-2} sec^{-1}, Missouri University Research Reactor, Columbia Missouri) to produce 367 μCi of ^{171}Er immediately postirradiation. Likewise, a 400-mg ^{153}Sm-radiolabeled tablet was prepared from a mixture of dicalcium phosphate, samarium oxide, and magnesium stearate (89.25:0.75:10.00, w/w). This tablet was irradiated for 15 sec to afford 77 μCi of ^{153}Sm immediately following irradiation. Phantom counting experiments began 18 hr after neutron bombardment and were completed by 36 hr postirradiation.

Dual-Labeled Tablet for in Vivo *Study*

A model bilayer tablet was prepared for an *in vivo* study which evaluated a dual-labeled erbium/samarium delivery system. A 300-mg mixture of dicalcium phosphate, erbium oxide, samarium oxide, and magnesium stearate (88.23:1.70:0.07:10.00, w/w) was precompressed (100 lb, 9.5-mm-diameter concave punch, Carver press) to form half of the bilayer tablet. This layer of the bilayer tablet was intended to remain intact throughout the gastrointestinal (GI) tract. Formation of the second layer of the bilayer tablet involved addition of a 300-mg mixture of anhydrous lactose, samarium oxide, and HPMC (92.73:0.27:7.00, w/w) to the first precompressed layer. The bilayer tablet was completed

[1] Division of Medicinal Chemistry and Pharmaceutics, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536-0082.

[2] To whom correspondence should be addressed.

[3] Department of Radiology, UKMC, and Department of Nuclear Medicine, VAMC, Lexington, Kentucky.

[4] Glaxo Inc., 5 Moore Drive, P.O. Box 13398, Research Triangle Park, North Carolina 27709.

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by direct compression on a Carver press at 4000 pounds. The second layer of the tablet was designed to undergo slow *in vivo* erosion over approximately a 2-hr period. Radiolabeling of the bilayer tablet was completed by neutron bombardment (Columbia, Missouri) for 50 sec to produce 245 μCi of ^{171}Er and 85 μCi of ^{153}Sm immediately postirradiation. Scintigraphic studies began 18 hr following neutron activation.

Study Design and Data Analysis

All experiments were performed using a large-field of view (LFOV) gamma camera (Siemens Basicam, Chicago, IL) equipped with a general purpose medium energy parallel hole collimator. Data were acquired in a dual-isotope acquisition as a 64 × 64 matrix and stored on computer (IBM AT, Cardiac Medical Systems Nuclear Medicine Software, Automatic Computing Engine, Springfield, WI) for future analysis.

In Vitro *Imaging Procedure: Phantom Counting of Single-Labeled Tablets*

Radioactive ^{171}Er and ^{153}Sm samples were suspended 6 cm apart in a water-filled lucite tank and positioned beneath a gamma camera. The ^{171}Er energy window was set to the 308-keV gamma ray and window width was decreased from 25 to 5% in 5% increments. The ^{153}Sm energy window was calibrated for the 103-keV gamma ray, and the window width was varied as described for the ^{171}Er channel. Cumulative counts over a 60-sec interval were acquired for sample depths of 0–3, 5–8, 10, 12, 14, and 16 cm. This procedure was repeated to provide duplicate readings.

The analysis of the phantom study required a region of interest (48 pixels) to be drawn around each sample image; all counts were decay corrected. The erbium-171 contribution into the ^{153}Sm window was reported as the number of ^{171}Er decay events detected in the samarium window divided by the ^{171}Er decay events observed in the erbium window.

Determination of ^{171}Er and ^{153}Sm Spatial Resolution

Information regarding possible limits of resolution when erbium- and samarium-labeled dosage forms reach close spatial proximity within the body was determined by suspending ^{171}Er and ^{153}Sm samples in a water tank at a constant depth (4 cm). The distance between the two samples was decreased from 4 to 1 cm in 1-cm increments. The ^{171}Er window was set at 308 keV and the ^{153}Sm window was set at 103 keV; a 10% window width was used for both energy windows. Analysis of this data examined the minimum distance between the two tablets at which image resolution was still maintained. Image resolution was defined as the ability to visualize enhanced computer images as discrete ^{171}Er and ^{153}Sm samples on the computer monitor.

Radionuclidic Purity Determinations

The radionuclidic purity of all samples was determined using a high-resolution Ge(Li) detector interfaced with a multichannel analyzer (14). All samples exhibited greater than 99% radionuclidic purity, with ^{24}Na and trace ^{154}Eu as the principal impurities.

In Vivo *Imaging of Dual-Labeled Bilayer Tablet*

In a feasibility study for dual-labeled ^{171}Er/^{153}Sm delivery systems, a bilayer tablet was orally administered to two human volunteers at 18 hr postactivation. At the time of dose administration each dual-labeled tablet contained 47 μCi of ^{171}Er and 65 μCi of ^{153}Sm. Subject 1 had fasted 8 hr and subject 2 ingested the tablet after a light lunch consisting of a chicken salad sandwich, tossed salad, and skim milk (250 ml). The analyzer energy settings were adjusted to 308 keV with a 15% window for the ^{171}Er, and the ^{153}Sm was calibrated at 103 keV with an 8% window. Following dose administration, each subject lay supine beneath a gamma camera and was imaged continuously for the first 45 min post dose administration. Thereafter, dynamic anterior imaging was continued for 15 min of every half-hour. Subjects were allowed to ambulate freely for 15 min while not being imaged. Since this was a preliminary study, this imaging schedule was stopped after 14 and 9 hr post dose administration for subjects 1 and 2, respectively.

Data analysis of this study examined the gastrointestinal locus of each radioisotope versus time and the relative release of the ^{153}Sm from the slow-eroding portion of the tablet with respect to the nondisintegrating tablet layer.

RESULTS AND DISCUSSION

The primary objective of this investigation was to describe methodology and radiolabeling procedures for dual-label scintigraphy studies which use the neutron-activated isotopes erbium-171 and samarium-153. Table I lists the primary gamma rays and corresponding percentage abundances of these two isotopes (19). Optimal energy settings for such dual-label studies should detect the 103-keV gamma ray of ^{153}Sm and the 308-keV gamma ray of ^{171}Er as (i) these gamma rays are the most abundant, and (ii) there is no direct energy overlap between the two gamma rays. Because of the intrinsic compton scatter of the high-energy ^{171}Er gamma ray, it is necessary to characterize the compton contribution from ^{171}Er into the lower-energy ^{153}Sm window.

Results from the *in vitro* study which evaluated limits of spatial resolution between ^{171}Er and ^{153}Sm are shown in Figs. 1A–D. The criteria for resolution were defined as the ability to visualize each radiolabeled tablet as a discrete image on the computer monitor. These results indicated that quantitative image resolution was achieved for samples 3 cm apart (Figs. 1A and B), while figures 1C and D demonstrated that it was possible to subjectively distinguish the two isotopes at a distance of 1 cm. It should be noted from these images that ^{171}Er is visible in the ^{153}Sm channel (Figs. 1B

Table I. Major Gamma Rays and Percentage Abundances of ^{171}Er and ^{153}Sm

Radioisotope	Half-life (hr)	Energy (keV)	Percentage abundance
^{171}Er	7.52	111.6	20.50
		295.9	28.90
		308.3	64.40
^{153}Sm	46.7	69.7	4.85
		103.2	28.02

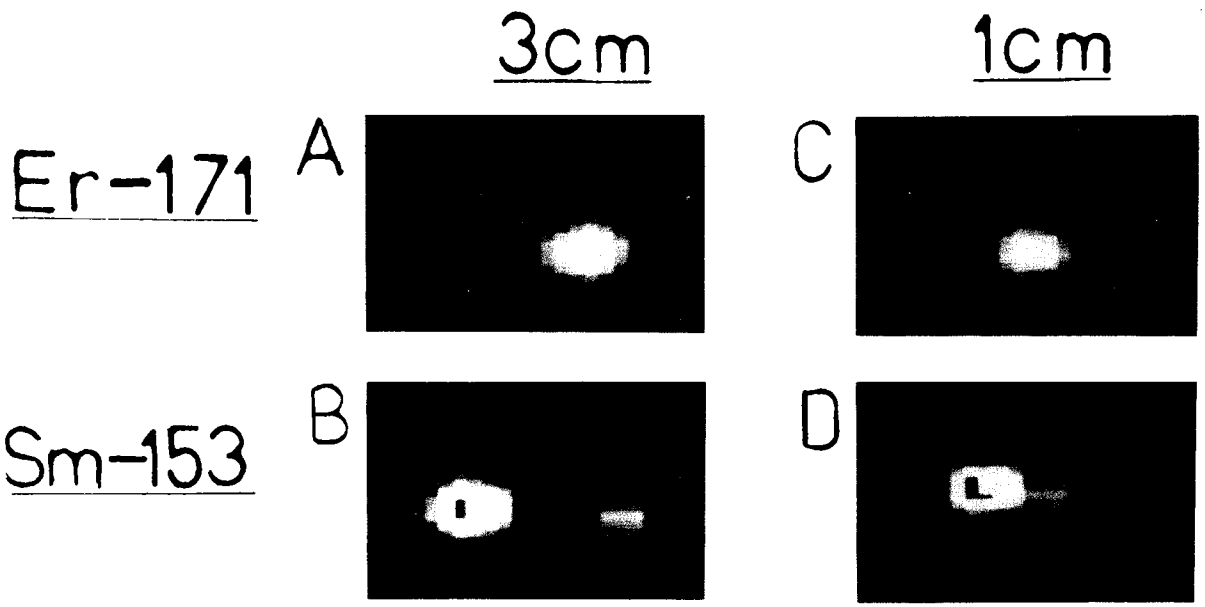

Fig. 1. Spatial resolution between erbium-171 (Er-171) and samarium-153 (Sm-153) samples at a constant depth (4 cm) in water. Analyzer settings were 308 keV with a 10% window and 103 keV with a 10% window for Er-171 and Sm-153, respectively. The Er-171 channel is represented by A and C, and the Sm-153 channel is represented by B and D. The distance between the two samples in A and B is 3 cm, and the samples in C and D are 1 cm apart. Note that the Er-171 sample is visible in the Sm-153 channel (B and D), but image resolution is still feasible. The Sm-153 sample is not observed in the Er-171 channel (A and C).

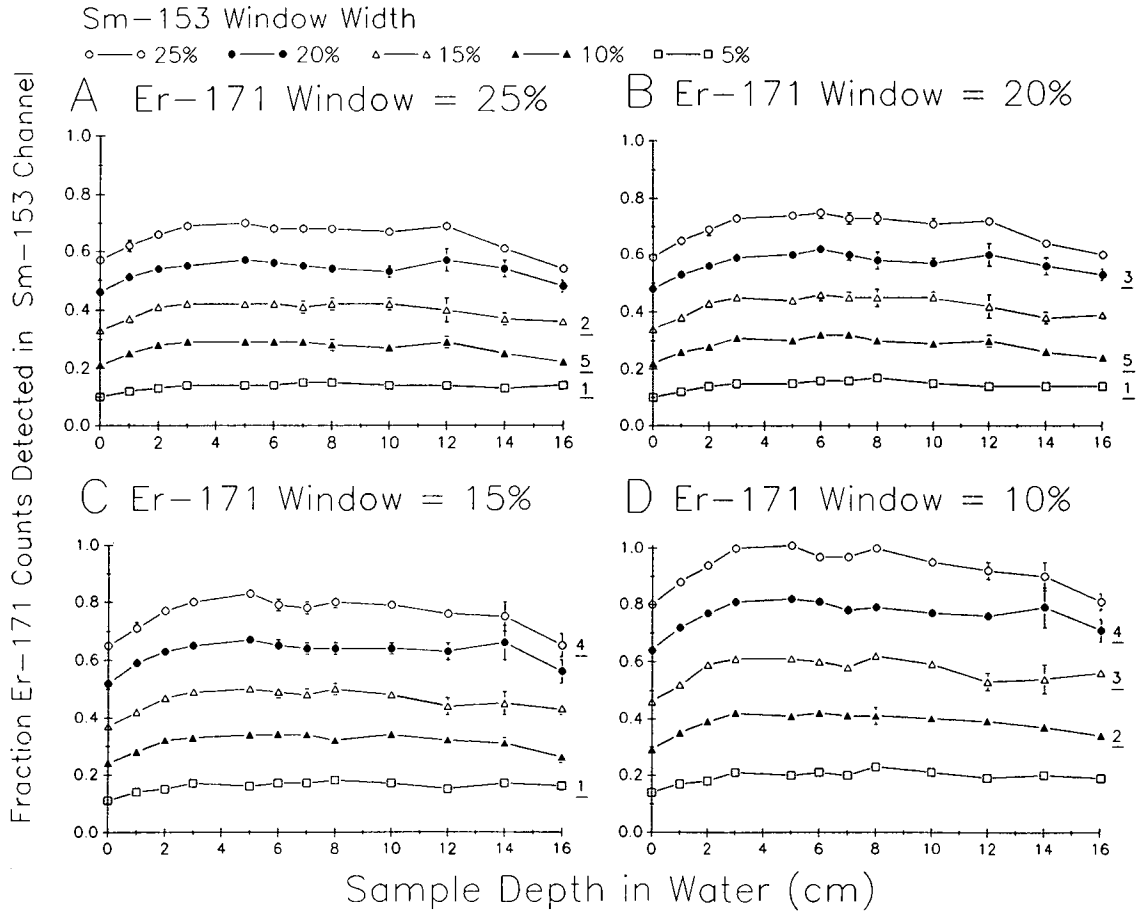

Fig. 2. Fraction of Er-171 decay events observed in both the Sm-153 channel (103 keV) and the Er-171 channel (308 keV) as a function of depth in water. Window widths for the Sm-153 channel were adjusted to 25% (open circles), 20% (filled circles), 15% (open triangles), 10% (filled triangles), and 5% (open squares) while maintaining a constant Er-171 window. (A) Er-171 window = 25%. (B) Er-171 window = 20%. (C) Er-171 window = 15%. (D) Er-171 window = 10%. 1–5 indicate that these line comparisons were not significantly different.

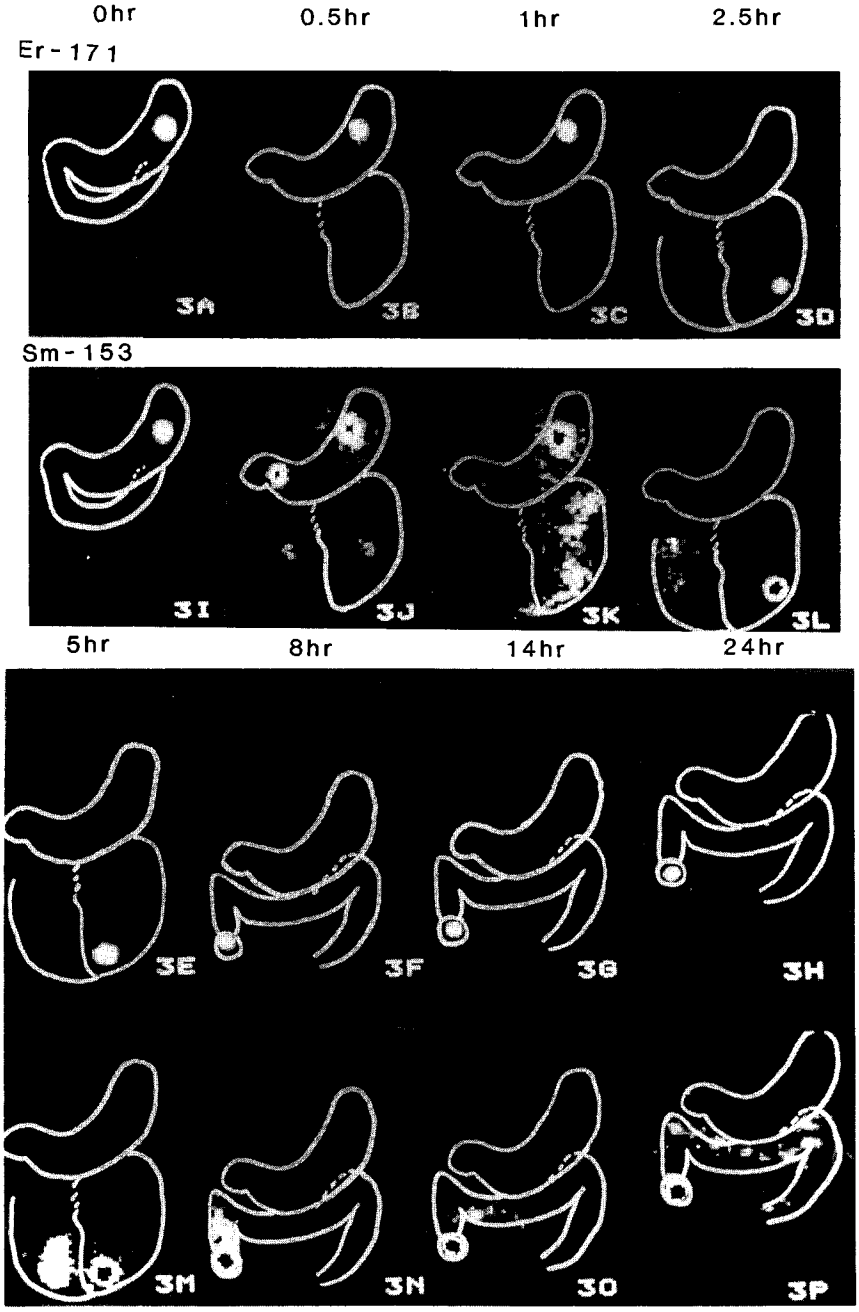

Fig. 3. Fasted subject number one gastrointestinal transit of the dual-labeled bilayer tablet. Figs. 3A–H show the non-disintegrating ^{171}Er tablet half and figs. 3I–M represent the disintegrating tablet portion labeled with ^{153}Sm. Refer to the text for a description of the scintigraphic images.

and D) because of compton scatter contribution, but the compton scatter does not interfere with the ability to determine spatial location of the dosage form.

Figures 2A–D quantitate the compton scatter contribution of ^{171}Er into the ^{153}Sm window for range of window-width settings and variable attenuation conditions. The acquisition conditions for a dual-label study of this type should (i) minimize the ^{171}Er compton scatter and (ii) maintain this contribution at a relatively constant level for the range of attenuation conditions which exist in the body.

Based on the criteria of constant and minimum ^{171}Er scatter, the data from Fig. 2 suggest that optimal analyzer settings are a wide ^{171}Er window and a narrow ^{153}Sm window. Statistical comparison of the average fraction of ^{171}Er counts detected in the ^{153}Sm window for each window width indicated that all settings were significantly different (paired t test, $P < 0.01$) with the exceptions noted in Figs. 2A–D. Thus, the results in Fig. 2 indicate that adequate analyzer settings can use a 5 to 10% ^{153}Sm window width and an ^{171}Er window of 15 to 25%. These settings provide a minimum and

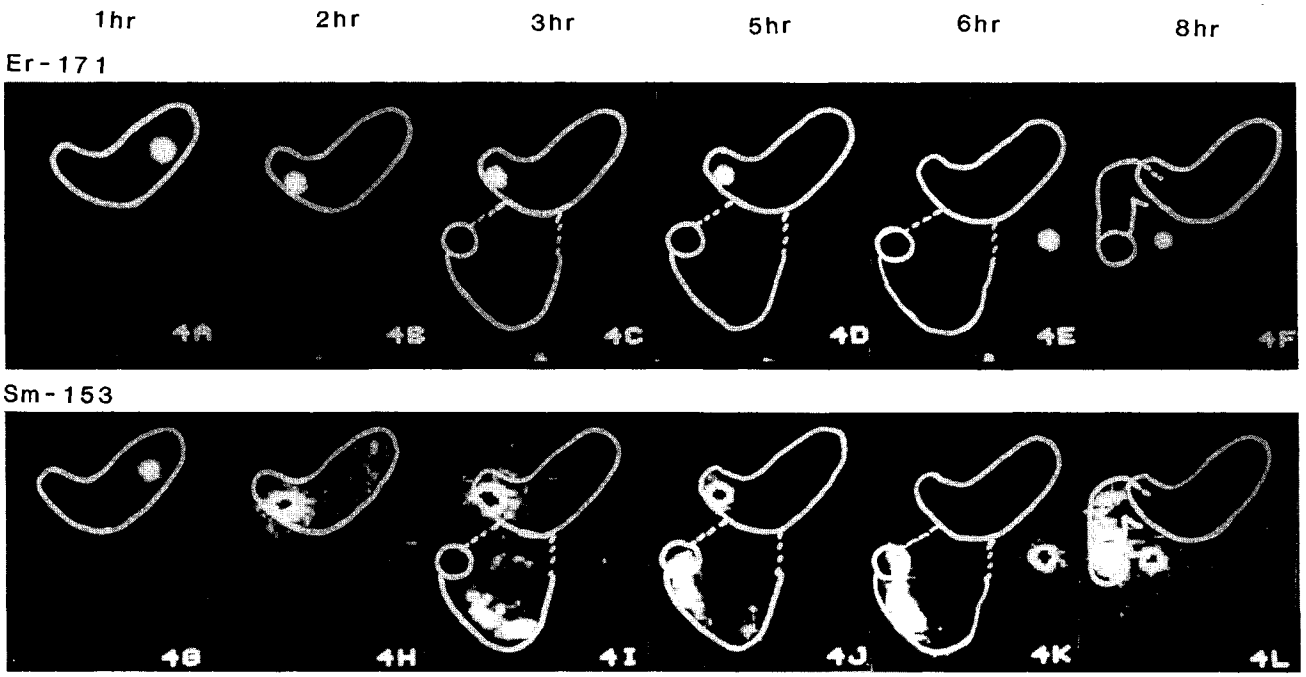

Fig. 4. Gastrointestinal transit of the dual-labeled bilayer tablet in non-fasted subject number two. Figs. 4A–F show the non-disintegrating ^{171}Er tablet half and figs. 4G–L represent the ^{153}Sm labeled portion which was designed to erode slowly. Refer to the text for a description of the scintigraphic images.

relatively constant ^{171}Er compton scatter contribution of approximately 15%. Though these window settings are adequate, we typically use an ^{171}Er window of 15% (308 keV) and a ^{153}Sm window of 8% (103 keV) for *in vivo* imaging.

The results from the *in vivo* study which evaluated the dual-labeled ^{171}Er/^{153}Sm bilayer tablet are shown pictorially in Fig. 3 for fasted subject 1 and in Fig. 4 for fed subject 2. The scintigraphic images indicate that the nondisintegrating portion can be observed in the ^{153}Sm channel due to the compton scatter of ^{171}Er and the trace levels of ^{153}Sm. However, this compton scatter into the ^{153}Sm window does not interfere with determining the gastrointestinal transit of either portion of the bilayer tablet.

Thus, for the fasted subject 1 (Figs. 3A–P) it was observed that the erodible HPMC/lactose portion (^{153}Sm) separated from the nondisintegrating half (^{171}Er) at 30 min postdose (Figs. 3B and J) and was subsequently observed to empty from the stomach at 39 min. Complete disintegration of the HPMC/lactose was apparent by 1 hr as indicated by the extensive dispersion in the proximal small intestine (Fig. 3K); the nondisintegrating ^{171}Er portion remained in the fundus of the stomach at 1 hr (Fig. 3C). Gastric emptying of the nondisintegrating half was observed at 92 min. The remainder of this study was characterized by collection and concentration of ^{153}Sm at the distal small intestine (Figs. 3L and M). The nondisintegrating half moved more slowly through the proximal small intestine, with little change in position at the distal jejunum from 2.5 through 5 hr (Figs. 3D and E). After the dinner meal at 7 hr postdose, the ^{171}Er nondisintegrating portion moved rapidly to the cecum and resided there through 24 hr postdose (Figs. 3F, G, and H). The ascending colon was observed at 8 hr from the ^{153}Sm images (Fig. 3N) with consistent filling into the transverse colon at 14 hr (Fig. 3O). The entire colon was outlined by ^{153}Sm distribution at 24 hr postdose (Fig. 3P).

Predictably, the transit behavior of the dual-labeled bilayer tablet was very different in the nonfasted subject, 2 (Figs. 4A–L). The nondisintegrating half remained in the stomach through the 5-hr imaging sequence (Figs. 4A–D). Likewise, the HPMC/lactose (^{153}Sm) half demonstrated an initial quiescent period postdosing where no release of ^{153}Sm was observed through 2 hr (Figs. 4G and H). However, at 3 through 6 hr, continual release of ^{153}Sm with subsequent collection in the distal small intestine was noted (Figs. 4I–K). The nondisintegrating (^{171}Er) portion emptied from the stomach between 5.5 and 6 hr and was located in the proximal small intestine at 6 hr (Fig. 4E). The ^{153}Sm released from the erodible portion of the tablet showed collection at the distal ileum with some indication of cecum filling at 6 hr (Fig. 4K). At 8 hr postdosing (following meal ingestion at 7 hr) there was significant ^{153}Sm activity in the ascending colon and hepatic flexure (Fig. 4L), while the nonerodible half resided in the ileum at 8 hr (Fig. 4F).

Although the discussion of these results do not quantitate the release rate of ^{153}Sm from the tablet, these data provide significant insight regarding quantitative gastrointestinal transit times of a bilayer tablet when administered under a fasted and nonfasted condition. More importantly, the results demonstrate the ability to evaluate simultaneously the behavior of two separate and dissimilar regions within the same unit dose by using the neutron-activated isotopes erbium-171 and samarium-153.

SUMMARY

The feasibility of dual-label scintigraphic studies which

use the neutron-activated isotopes erbium-171 and samarium-153 is described. Experimental details are provided to correct and minimize the compton scatter contribution of ^{171}Er into the lower-energy ^{153}Sm window.

The results from this study demonstrate that this dual-label procedure is sensitive enough to monitor simultaneously the behavior of two discrete regions of the same unit dose in the gastrointestinal tract of man.

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