Magnetic Resonance in Medicine 48:255–261 (2002)

Gastrointestinal Transit Times in Mice and Humans Measured With ^{27}Al and ^{19}F Nuclear Magnetic Resonance

Roman Schwarz, Armin Kaspar, Joachim Seelig,* and Basil Künnecke

Gastric emptying and gastrointestinal (GI) transit times in mice and humans were monitored noninvasively by using ^{27}Al and ^{19}F nuclear magnetic resonance (NMR). Al^{3+} bound to ion-exchange resin and perfluorononane were administered orally as selective and specific markers for the stomach and the entire GI tract, respectively. ^{27}Al- and ^{19}F-MR spectroscopy (MRS) was employed to follow quantitatively boli of the mixed markers in awake, fed mice over a period of 48 hr. The selectivity of the markers was confirmed by whole-body ^{1}H-, ^{27}Al-, and ^{19}F-MRI of anesthetized mice. Gastric emptying in humans was also monitored with ^{27}Al-MRS of aluminum-loaded ion exchange resin. GI transit was assessed by ^{19}F projection imaging of pharmaceutical capsules tagged with perfluorononane. Quantitative analysis of the MR data revealed that gastric emptying in humans proceeded linearly, whereas in mice an exponential decay was observed. This difference is explained by the respective feeding patterns of humans and mice. Humans usually achieve nearly complete gastric emptying before each meal. In contrast, very short delays between successive food intakes in small animals result in successive dilution of the stomach contents. For stomach emptying in mice the exponential decay constant was 74 min, whereas the half-time of the linear gastric emptying in humans was 30 min. Magn Reson Med 48: 255–261, 2002. © 2002 Wiley-Liss, Inc.

Key words: nuclear magnetic resonance; contrast agent; gastrointestinal tract; transit times; perfluorononane; aluminum-resin

Gastrointestinal (GI) dwell and transit times have been recognized as crucial factors in the oral administration of solid drugs, because precise knowledge of the anatomical location of drug release in situ allows the pharmaceutical potential of oral drugs to be maximized (1). Moreover, GI diseases often elicit changes in GI transit time, which are of diagnostic value and provide a means of following up therapy of various diseases (2).

A variety of methods have been applied for assessing GI transit times, motility, and drug release. Most prominent are the X-ray (3) and scintigraphic techniques (4), which have been used to monitor orally ingested capsules containing radioopaque material or gamma-emitters. On the other hand, noninvasive techniques such as ultrasound (5), metal detectors (6), magnetic field detectors (7), and dyes (8) have been used to avoid the adverse effects of ionizing radiation. However, all these methods have been severely restricted due to intrinsic constraints such as low temporal or spatial resolution, lack of complementary anatomical information, or incomplete spatial information. As an alternative, MRI techniques could, in principle, offer a convenient, noninvasive modality for monitoring GI transit that does not suffer from these limitations. Unfortunately, despite its success in clinical diagnosis, ^{1}H-MRI has generally failed in tracing small objects in the bowel because of large and intricate local signal changes. Specific tagging of capsules with conventional contrast agents based on the alteration of the magnetic susceptibility has not mitigated this problem (9). Conversely, ^{19}F-MRI of fluorinated agents has been shown to provide excellent selective contrast in images of the murine GI tract (10,11). Due to the lack of endogenous fluorine-containing metabolites in soft tissues, the ^{19}F-MR signal can be assigned exclusively to the fluorinated contrast agent present inside the GI tract. The intensity of the ^{19}F-MR signal is therefore a measure of the amount of contrast agent present in the GI tract. By monitoring the intensity variations of the ^{19}F signal, the flow of the contrast agent out of the GI tract can be followed noninvasively.

The routine procedure for studying the gastric emptying of the solid phase involves labeling ion-exchange resin with radioactive isotopes, which are detected by γ-scintigraphy (12,13). In principle, equivalent assessments using MR methods should be feasible if the radioisotopes are substituted with magnetic active nuclei. It has been shown that ^{27}Al-MRI and ^{27}Al-MR spectroscopy (MRS) of Al^{3+} released from aluminum-containing antiacidics can be used to selectively delineate the stomach (14). ^{27}Al has a quadrupolar moment that leads to quadrupole splittings that normally broaden the resonance beyond detection. However, if the ^{27}Al nucleus experiences a highly symmetric environment, the quadrupolar splitting vanishes and the Al^{3+} resonance has a linewidth of only a few Hz. This is the case for Al^{3+} in aqueous solution at a pH below 3.5—a condition typically found in the stomach but not in the remainder of the GI tract (14). Al^{3+} readily binds to cation-exchange resins in analogy to the radioisotopes used for γ-scintigraphy. Interestingly, such resin-bound Al^{3+} has MR properties similar to those of free aluminum ions (15). Moreover, aluminum has a very low natural occurrence in biological tissues. By taking advantage of these properties, Al-loaded resin can be used as a selective marker for the gastric contents

Department of Biophysics, Biocenter, University of Basel, Basel, Switzerland.

Grant sponsor: Swiss National Science Foundation; Grant number: 31.49758.96; Grant sponsor: Doerenkamp Zbinden Foundation.

Presented in part at the 7th Annual Meeting of ISMRM, Philadelphia, 1999.

B. Künnecke's present address is F. Hoffmann-La Roche, Magnetic Resonance Imaging and Spectroscopy, PRBD-M, Bldg. 68/05A, CH-4070 Basel, Switzerland.

*Correspondence to: Prof. Joachim Seelig, Dept. of Biophysics, Biocenter, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.
E-mail: Joachim.Seelig@unibas.ch

Received 16 October 2001; revised 14 March 2002; accepted 14 March 2002.
DOI 10.1002/mrm.10207
Published online in Wiley InterScience (www.interscience.wiley.com)

In the present work, the concept of selective ^{19}F and ^{27}Al markers was applied to quantitative MR assessments of gastric and GI emptying in mice and a human subject.

MATERIALS AND METHODS

Chemicals

Perfluorononane with a nominal purity of 97% was purchased from Aldrich Chemical Co. (Switzerland). The cation-exchange resin used was based on a sulfonated diethenylbenzene polymer. Two different makes were employed: Dowex Marathon C (Sigma, Buchs, Switzerland), with a particle size of 30–40 mesh, was used for human application; for the mice, Resonium A (Sanofil Winthrop, Münchenstein, Switzerland), in powder form, was administered.

Preparation of the Contrast Agents

Preparation of the Combined $^{19}F/^{27}Al$ Contrast Agent for Animal Use

Resonium A was suspended in a saturated solution of $AlCl_3*6H_2O$ for 24 hr. Subsequently, it was washed with 0.1 M HCl and twice with pure water, both added in at least 10-fold excess. For storage, the aluminum-loaded Resonium A was dried with air. Prior to the gavage, 100 mg of dry Resonium A was soaked in 500 μl water for at least 24 hr. The supernatant was then discarded and 100 μl of perfluorononane (with 1% detergent) were added. The two components were briefly mixed with a spatula and immediately administered to the animal.

Preparation of the Aluminum Contrast Agent for Human Use

Dowex Marathon C (60 g dry weight) was suspended in a solution of 90 g $AlCl_3*6H_2O$ in 1 liter of water for 24 hr under constant stirring. Prior to its administration, the resin was washed with 1 liter of 0.1 M HCl, and twice with 1 liter of pure water.

Preparation of the ^{19}F-Filled Capsule for Human Use

A capsule with a length of 22 mm and a diameter of 7 mm (size 0) was made of chemically stable and biologically inert polychlortrifluorethylene (PCTFE) and was filled with 350 μl of perfluorononane (C_9F_{20}). The capsule, including its filling, had a specific density of 2.0 g/ml.

GI Assessments in Mice

Eight well-fed female BL/6 mice (21–32 g) were used. The animals received a standard laboratory mice chow and drinking water ad libitum. Prior to the first MR examination, each mouse was given a gavage of 0.3 ml of the $^{19}F/^{27}Al$ contrast agent prepared as described above. Immediately after the gavage the animal was placed in prone position in a narrow tube, which was inserted into the $^{19}F/^{27}Al$ resonator used for the MR examinations. The tube allowed the animal to move back and forth within the sensitive volume of the resonator, but hindered the animal from turning. MR spectra were routinely acquired every and intestinal emptying were expressed as the time constant of mono-exponential decays fitted to the experimental data.

In order to assess the location of the contrast agent, two of the eight animals were anesthetized immediately after the gavage with 1.5% isoflurane in N_2O/O_2 (3:1) and then placed in a prone position in the same resonator as described above. Thirty minutes after the gavage, ^1H-, ^{27}Al-, and ^{19}F-MR images were acquired without repositioning the animals. The animals were allowed to wake up and were reanesthetized 3 hr later for reexamination.

GI Assessments in Man

All measurements were performed within the guidelines of the local ethics committee.

^{19}F Measurements

A male volunteer who had fasted overnight was asked to swallow a capsule filled with ^{19}F contrast agent while he lay inside the magnet. The abdominal region was repeatedly assessed with ^1H- and ^{19}F-MR in measuring periods of approximately 30 min duration. The first assessment was performed immediately before, and the subsequent ones at 1¾ hr, 3¼ hr, 5 hr, 10½ hr, 55 hr, and 75 hr after ingestion of the capsule. The first 5 min of each session were used for ^1H-MRI-guided (re)positioning of the volunteer; the rest of the session was spent on capsule tracking by ^{19}F-MR. While the capsule was in the stomach, the volunteer was assessed in the supine position. Thereafter, while the capsule was moving through the GI tract, the volunteer was examined in the prone position. Between subsequent assessments, the volunteer was allowed to stand up, walk around, and eat according to his usual habits.

^{27}Al Measurements

After ingestion of 5 g wet weight of aluminum-loaded resin, ^{27}Al spectra were acquired every 5 min. The volunteer was in the prone position and the surface coil was positioned under the gastric region as verified by conventional ^1H-MRI. In a separate experiment, ^{27}Al images were recorded 10, 20, and 40 min after the ingestion of 49 g (wet weight) Al-loaded resin. After the MR measurements, stool was collected to recover the resin.

MRI and MRS

In Vivo MRS and MRI of Mice

^{19}F- and ^{27}Al-MR spectra were acquired on a Bruker Biospec 70/20 (7 Tesla, 20-cm horizontal-bore magnet). An in-house-built Helmholtz coil with a diameter of 45 mm was used for ^{27}Al-NMR signal excitation and detection. A birdcage resonator with an inner diameter of 30 mm was used for ^{19}F-NMR. The birdcage coil was located between the pair of Helmholtz coils, with its axis orthogonal to the orientation of the latter coils. ^{19}F spectra were recorded using the following acquisition parameters: 90° pulses (94 μs), 6000 Hz spectral width, 1024 data points, a total recycle delay of 2.1 s, and eight averages. Only the CF_3

folded signals of the CF_2 groups were eliminated by a set of high-order analog and digital filters. Aluminum spectra were recorded using the following acquisition parameters: 90° pulses (130 μs duration), 12 kHz spectral width, 512 data points, a total recycle delay of 37 ms, and 1500 averages.

For ^{1}H imaging, the ^{19}F resonator was tuned to the proton frequency by the addition of a copper shield around the resonator (for ^{19}F and ^{27}Al imaging this shield was removed). Coronal projections for ^{19}F and ^{27}Al, and 1-mm slices for proton MRI were obtained over a field of view (FOV) of 6 × 6 cm^2 using the following parameters: a rapid acquisition with relaxation enhancement (RARE) sequence (16) with a 128 × 128 matrix, TR/TE = 1297/75 ms, and a RARE factor of 16 for ^{1}H MRI; a single-shot RARE sequence with a 128 × 128 matrix, TR/TE = 2000/439 ms, and two averages for ^{19}F-MRI; and a gradient-echo sequence with a 64 × 64 matrix, TR/TE = 8.2/2.5 ms, and 1000 averages for ^{27}Al-MRI.

In Vivo ^{27}Al MRS and MRI of Man

^{27}Al-MR spectra were acquired on a 1.5 Tesla Siemens Magnetom Vision (Siemens, Erlangen) equipped with an additional broadband channel. An in-house-built, two-turn surface coil with an inner diameter of 13.5 cm was used for ^{27}Al signal excitation and detection (15). ^{27}Al spectra were recorded using the following acquisition parameters: pulses of 250 μs duration (adjusted in intensity to provide maximum signal), 1000 averages, and a total recycle delay of 98 ms. ^{27}Al images were acquired by using a standard fast low-angle shot (FLASH) sequence with the following acquisition parameters: 60° flip angle, TR/TE of 10.6/3.2 ms, 64 × 128 pixels covering an FOV of 15 × 15 cm^2, and 300 averages, which resulted in a total scan time of 3½ min.

In Vivo ^{19}F-MR Projection Imaging of Man

MR measurements were performed on the same clinical MR scanner as described above using an in-house-built ^{19}F surface coil integrated into the patient bed, and a commercial ^{1}H flexi-coil placed opposite to the ^{19}F coil. The 3D spatial location of the tracer capsule was determined by using projections obtained with a modified fast imaging with steady precession (TrueFISP) sequence (17), from which the slice and phase gradients had been removed. A total of 128 scans for each of the three spatial directions were averaged interleaved with a TR of 5.9 ms, resulting in a time resolution of 2.26 s. A spatial resolution of 2.7 mm was obtained by acquiring 128 data points over an FOV of 35 cm. Conventional anatomical ^{1}H-MR images were obtained with a multislice TrueFISP sequence. Coronal views with a slice thickness of 6 mm, FOV of 35 × 35 cm^2, and TR/TE set to 4.8 ms/2.3 ms were acquired into a data matrix of 256 × 256 points.

RESULTS

^{19}F and ^{27}Al Measurements in Mice

Figure 1a shows a coronal slice through the abdominal shows corresponding coronal projections made at the ^{27}Al and ^{19}F resonant frequency. All three images were acquired about 20 min after contrast agent administration, without repositioning the mouse between the different scans. Figure 1a delineates the anatomy of the abdominal region close to the ventral abdominal wall. Due to proton depletion caused by the perfluorinated contrast agent or the general lack of protons, the stomach and several loops of the bowel, as well as the lungs, were depicted as dark areas. ^{27}Al-MRI (Fig. 1b) detected a bright region at the location of the stomach. No other signal, with the exception of some scanner-specific noise at the upper fringe of the image, could be observed. Similarly, ^{19}F-MRI (Fig. 1c) also detected a region of strong signal intensity at the location of the stomach. However, this region has a caudal extension, which can be assigned to fluorine that has already left the stomach and has entered the duodenum. After the animal had been awake for 3 hr, it was reassessed with ^{1}H-, ^{27}Al-, and ^{19}F-MRI using the same acquisition parameters as in the first examination. The anatomical ^{1}H-MR image (Fig. 1d) shows few changes, aside from that caused by a slightly different positioning of the animal within the probe. However, the ^{27}Al signal has almost completely vanished (Fig. 1e) and the fluorine signal is distributed within different loops of the bowel (Fig. 1f).

In Fig. 2, representative in vivo ^{27}Al- (left stack) and ^{19}F-NMR spectra (right stack) acquired in an awake animal after administration of a bolus of contrast agent are plotted against time. Both time courses show distinct decreases of signal intensities over time. Since the ^{27}Al signal can only be observed while the contrast agent is in an acidic environment, the decay of the ^{27}Al signal (left stack) represents the stomach emptying. On the other hand, perfluorononane is metabolically inert and is not resorbed. The only way it can leave the body is by rectal excretion. Therefore, the decay of the ^{19}F signal represents the time course of emptying of the entire GI tract.

For a more quantitative analysis of gastric and GI emptying, the ^{27}Al- and ^{19}F-MR signals were integrated and plotted against time. Figure 3 shows the time courses of these signals in the same animal as used in Fig. 2. The circles (aluminum resonances) show the stomach emptying, and the diamonds (fluorine signal) indicate the emptying of the entire GI tract. The stomach emptying follows an exponential decay starting immediately after administration of the contrast agent. The release of the fluorine has a pronounced lag phase, which is also followed by an exponential decay. The ^{19}F-NMR signal does not return to zero because a small amount of perfluorononane remains in the stomach (verified by ^{19}F-MRI). This rest is completely eliminated after approximately 3 days (not shown). The experiment was repeated with a total of six animals. For stomach emptying, the exponential decay constants averaged $\tau = 74 \pm 17$ min, whereas for the GI transit the lag time until perfluorononane excretion started was 154 ± 24 min. However, the rates of gastric emptying, as well as the lag times, showed considerable interindividual variations. Large variations were also detected for repetitive measurements of the same animal. Nevertheless, the shapes of the curves were the same for all mice, except for two individuals that refused to eat. In those two cases, no

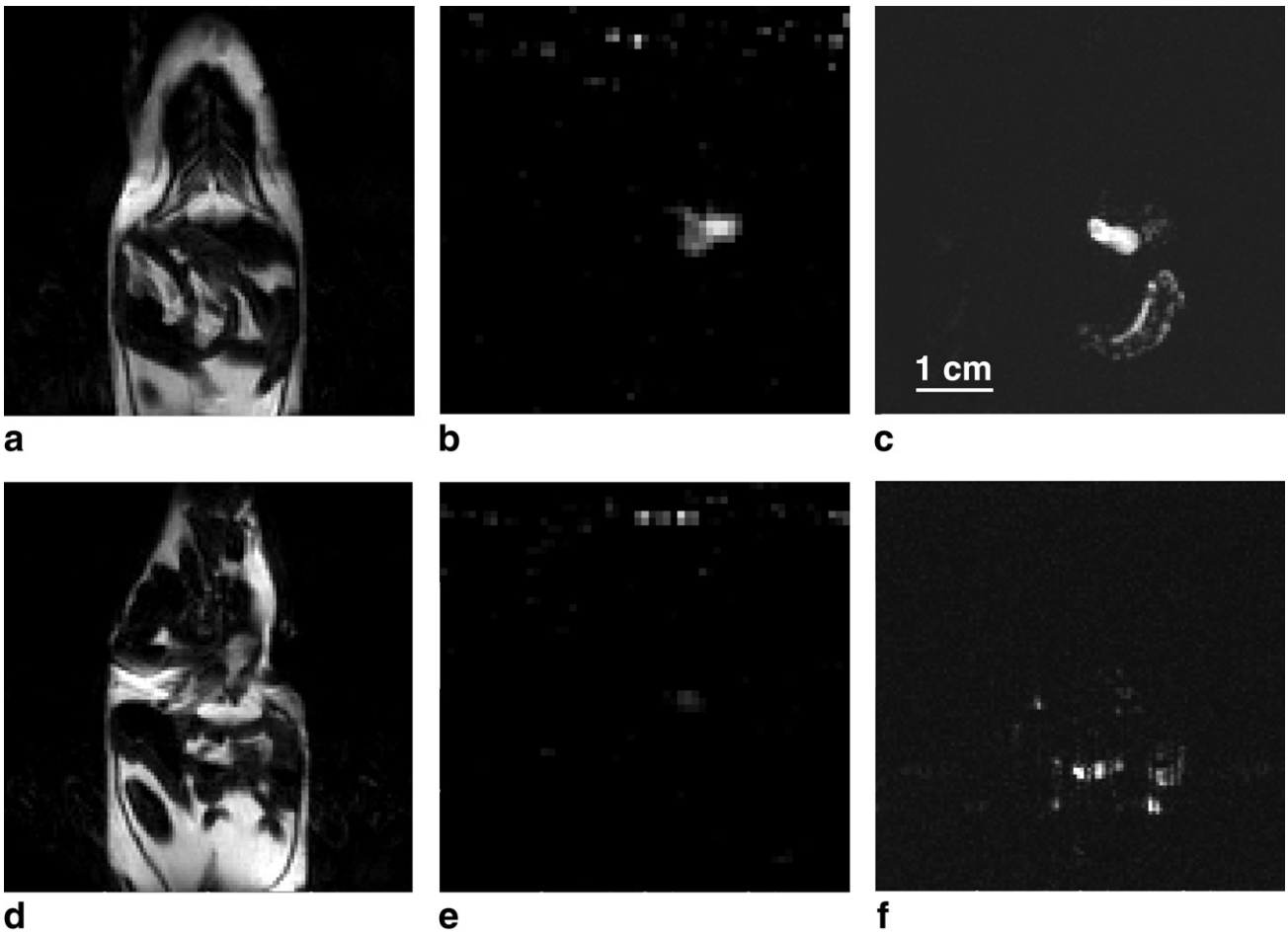

FIG. 1. Upper trace: (**a**) ^{1}H-, (**b**) ^{27}Al-, and (**c**) ^{19}F-MR images of the abdominal region of an anesthetized mouse acquired approximately 20 min after the gavage of ^{19}F/^{27}Al contrast agent. Lower trace: (**d**) ^{1}H-, (**e**) ^{27}Al-, and (**f**) ^{19}F-MR images of the abdominal region of the same mouse as above, but obtained from the reanesthetized animal, which had been allowed to move freely for 3 hr.

other hand, stomach emptying had taken place, but at a significantly slower rate.

Apart from signal decay over time, the ^{19}F resonances showed intricate line-shape variations that were attributed to macroscopic magnetic field inhomogeneities in the animal's bowel. There was a trend toward a shift to higher frequencies at later time points, suggesting that deconvolution of the observed line shape may provide additional information as to the current location and/or the environment of the perfluorinated contrast agent. However, because of its complexity this potential source of information was not further assessed in the present study.

^{27}Al Measurements in Man

GI assessments akin to those carried out with mice were also performed in the human subject. Figure 4 shows a coronal projection obtained by ^{27}Al-MRI of the abdominal region in man. The image was acquired 10 min after the ingestion of aluminum-loaded resin. A bright area is visible at the location of the stomach (as verified by ^{1}H-MRI; not shown); no signals were detected in other regions.

For a quantitative analysis of the gastric emptying, the

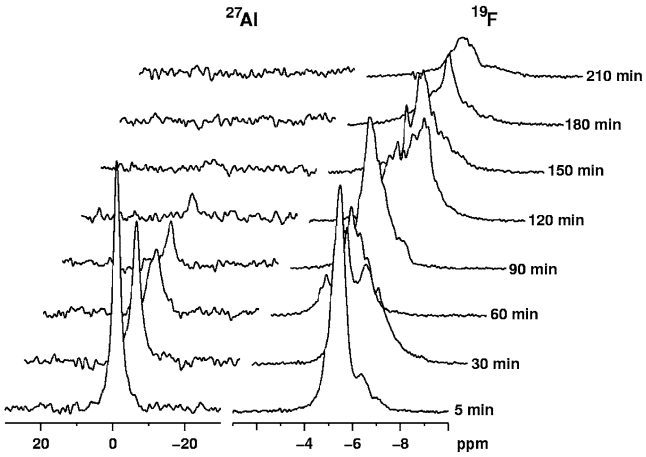

FIG. 2. Time course of in vivo ^{27}Al- (left) and ^{19}F-NMR spectra (right) obtained from an awake mouse after gavage of ^{27}Al/^{19}F contrast agent. The aluminum signal intensity represents pure gastric emptying, whereas the fluorine signal arises from both the stomach and the intestinal tract and represents the course of emptying of the entire GI tract. The decrease of the aluminum signal is significantly faster than that of the fluorine signal, which, in addition, shows a

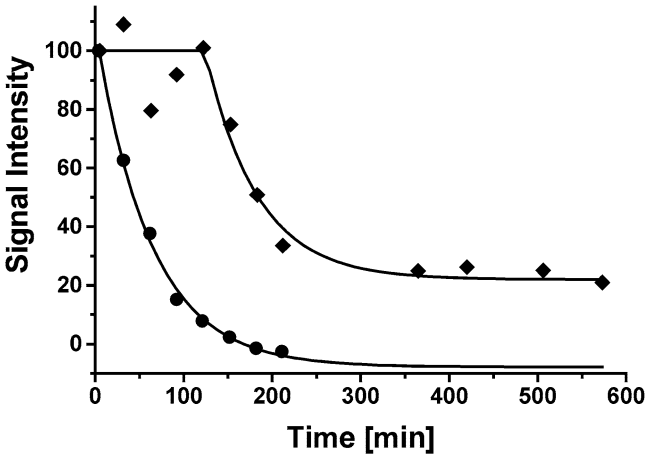

FIG. 3. The integrated signal intensities of the ^{27}Al- and ^{19}F-NMR resonances depicted in Fig. 2 as a function of time. The aluminum data (circles) show the stomach emptying, and the emptying of the entire GI tract is represented by the fluorine data (diamonds). The stomach emptying as described by ^{27}Al-NMR follows an exponential decay beginning immediately after administration of the contrast agent. The fluorine intensity remains approximately constant for the first 2 hr and then starts decreasing exponentially. The offset in the fluorine curve arises from a small amount of perfluorononane that remains in the stomach for approximately 3 days.

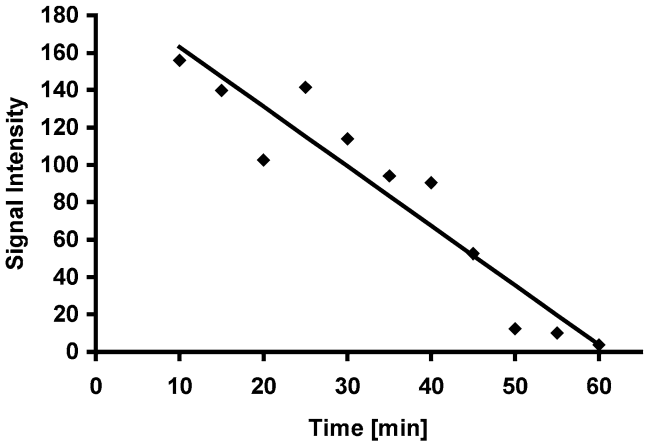

FIG. 5. The signal integral of ^{27}Al in a human stomach vs. time after ingestion of Al-loaded resin.

Figure 5 exemplifies a typical time course. A linear signal decrease can be observed. After 60 min the amount of resin inside the stomach was below the limit of detection. In light of the pH dependence of the ^{27}Al signal originating from ingested aluminum resin (as outlined above for mice), this decrease reflects the gastric emptying. If a linear decay is fitted to the time course, an emptying rate of 1.64%/min is obtained, which corresponds to a half-time of about 30 min.

^{19}F Measurements in Man

Emptying of the entire GI tract in man was assessed with ^{19}F-MR of perfluorononane in analogy to the measurements performed in mice. However, the perfluorononane was not given as a liquid; instead it was enclosed in an inert capsule. Figure 6 shows a typical coronal view of the abdominal region obtained shortly after ingestion of such a capsule. Due to the complete absence of protons, the capsule could be identified as a black bar (arrow) in the stomach, the latter being partially filled with gastric juice.

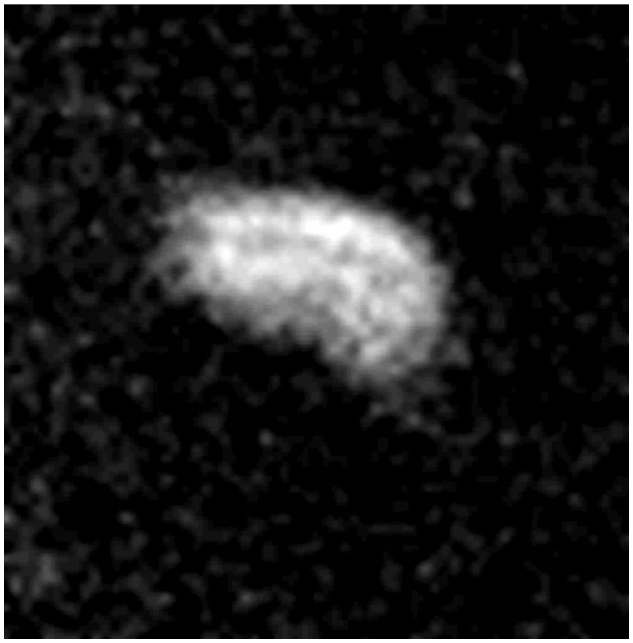

FIG. 4. Coronal projection of the abdominal region of a man, acquired by ^{27}Al-MRI. The bright region represents the stomach,

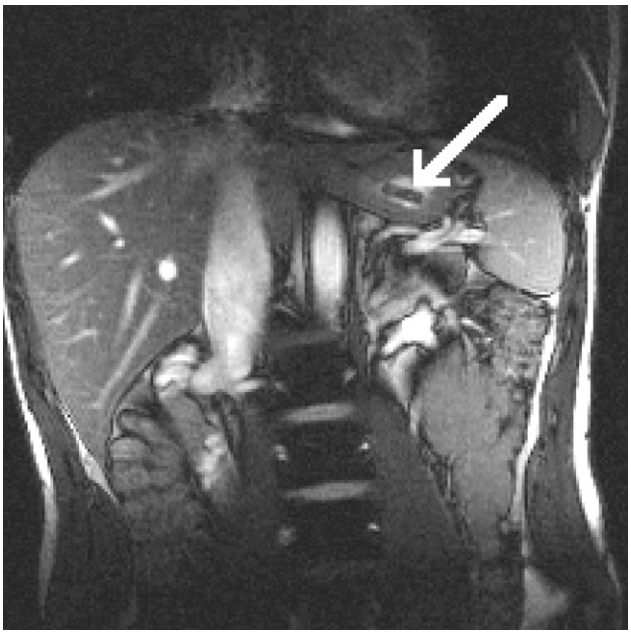

FIG. 6. Coronal view of the abdominal region of a volunteer obtained shortly after ingestion of a pharmaceutical capsule filled with perfluorononane. Due to complete proton depletion, the capsule

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.