In vivo evaluation of a colonic delivery system using isotope techniques

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SUMMARY
Aim: To evaluate, using isotope techniques, the in vivo effectiveness of a pH-dependent colonic delivery system.
Methods: In order to dispose of differently labelled substrates for measurement of orocaecal transit time, inulin-^{14}C-carboxylic acid was evaluated as an alternative substrate to inulin and lactose-^{13}C-ureide. Secondly, the time of release of ^{13}C- and ^{15}N-urea from the colonic delivery system was compared with the orocaecal transit time, measured using inulin and inulin-^{14}C-carboxylic acid. This study was repeated after a 2-week lactulose intake period.
Results: The orocaecal transit time determined using inulin-^{14}C-carboxylic acid (398 min) was not significantly different from the orocaecal transit time determined using inulin (420 min) or lactose-^{13}C-ureide (396 min). Before lactulose intake, the ^{13}CO_{2} excretion time was 358 min and the orocaecal transit times determined with the inulin-^{14}C-carboxylic acid and inulin breath test were 376 and 375 min respectively. After lactulose, the ^{13}CO_{2} excretion time was 383 min and orocaecal transit times were 354 min for inulin-^{14}C-carboxylic acid and 392 min for inulin. A highly significant correlation was found. Good agreement was found between the urinary ^{15}N excretion and the appearance of ^{13}CO_{2} in breath.
Conclusion: Isotope techniques provide an excellent non-invasive tool for the in vivo evaluation of a colonic delivery system.

INTRODUCTION
Stable isotopes have been widely applied in substrates for breath tests in order to evaluate gastro-intestinal functions such as gastric emptying and orocaecal transit time (OCTT), often in combination with parameters of digestion. Up to now, application of isotopically labelled substrates in the evaluation of colonic metabolism has been hampered because of difficulties encountered in delivering the substrates to the colon.

During the past decades, a large number of colon-specific drug delivery systems have been developed for colon-targeted drug delivery and therefore, it was investigated whether a colon-specific drug delivery system could be used to deliver labelled substrates to the colon. For this purpose, a variety of pharmaceutical approaches are available such as coating with pH-sensitive polymers, formulation of timed-release systems and the use of carriers that are degraded specifically by colonic bacteria. From our point of view, it was important to choose a colon delivery concept which starts to release its content immediately upon arrival in the colon (i.e. no sustained release) and which does not depend on the characteristics of the drug to be delivered so that it could be applied to a variety of substrates. Therefore, coating of a gelatin capsule with a pH-sensitive polymer was considered as the most appropriate strategy. The pH-dependent systems exploit...
the generally accepted view that the pH of the human gastrointestinal tract progressively increases from the stomach (pH 1–2 which increases to 4 during digestion) over the small intestine (pH 6–7) to the distal ileum (pH of 7–8).2 As it is possible that different pH levels exist in the parts of the gastrointestinal system of different individuals, it is important to evaluate the release pattern of the colon delivery system.

Whereas 

_in vitro_ evaluation of the release pattern of such pH-dependent systems can easily be performed, _in vivo_ evaluation of its performance in humans is much more difficult and has most often been performed with γ-scintigraphy.1 In this study, we suggest a novel non-invasive and easy-to-perform approach for the _in vivo_ evaluation of colonic delivery systems using breath test technology for measurement of OCTT.

Currently, hydrogen breath test using lactulose and inulin as substrates as well as 13C2-breath test using lactose-13C-ureide, have been validated and are routinely available.3–8

In this study, we preferred to include two labelled substrates (13C-urea and 15N-urea) in the polymer coated capsule and to compare the time at which they were released to the time of arrival in the colon of two substrates for measurement of OCTT (inulin and inulin-14C-carboxylic acid). Upon arrival in the colon, inulin-14C-carboxylic acid is metabolized to 14CO2 which can be measured in breath simultaneously with 13CO2 and H2. However, inulin-14C-carboxylic acid has not been validated before as a substrate for OCTT and therefore, the first part of the study consisted of a comparative evaluation of inulin-14C-carboxylic acid to the standard substrates lactose-13C-ureide and inulin.

In the second part, the performance of the colonic delivery system was evaluated by comparing the time of a rise in breath 13CO2 excretion and urinary 15N excretion with the time of increased 14CO2 and H2 excretion in breath. The study was repeated after a 2-week intake period of lactulose as this treatment influences the colonic pH and hence, may influence the release of the colonic delivery system.

**MATERIALS AND METHODS**

**Subjects**

None of the subjects had a history of gastrointestinal or metabolic disease or previous abdominal surgery (apart from appendectomy). The subjects were free of antibiotics or any other medical treatment influencing gut transit or intestinal flora for at least 3 months before the start of the study. The Ethical Committee of the University of Leuven approved the study and all subjects gave informed consent.

**Substrates**

13C-urea was obtained from Campro (Veenendaal, The Netherlands) and was used as such as well as for the synthesis of lactose-13C-ureide (synthesized according to the method of Schoorl9 as modified by Hofmann10). Raftilin HP (>99% inulin with a degree of polymerization between 5 and 60 and <0.5% glucose, fructose and sucrose) was obtained from Orafti (Tienen, Belgium) and inulin-14C-carboxylic acid was purchased from Amersham Biosciences (Uppsala, Sweden). Inulin-14C-carboxylic acid is made from inulin of chicory root. It is prepared by the condensation of 14C-cyanide with inulin followed by hydrolysis (CnH10O4n+2O5n+1(COOH)5; MW 5200 g/mol). [15N, 15N]-urea was obtained from Euriso-top (St Aubin, Cédex, France).

**Colonic delivery system**

The colonic delivery system consisted of a hard gelatin capsule (size 2) containing 75.6 mg of 13C-urea (equimolar amount as 500 mg lactose-13C-ureide), 8 mg of 15N-urea, 25 mg of riboflavin monophosphate (Certa, Braine l’alleud, Belgium), and 130 mg of lactose monohydrate (HMS Uitgeest, The Netherlands). The capsule was coated with a pH dependent film made up of cellulose acetophthalate (21% w/w) (Acros, Geel, Belgium) and diethylphtalate (Acros, Belgium) (15% w/w with respect to polymer).11 In order to investigate the pH dependent characteristics of the polymer coating, the release of a marker, riboflavin monophosphate, was monitored during _in vitro_ dissolution testing experiments in simulated gastric fluid (pH 1.2) (USP XXV) and in phosphate buffers (0.1 m) of pH 5.0, 6.0 and 7.0 using the USP XXV paddle method (100 rpm) at 37 °C. The concentration of the marker was quantified spectrophotometrically at 457 nm.

No trace of riboflavin monophosphate could be detected after 2 h in simulated gastric fluid indicating the gastric resistance of the polymer film, while the time to release 50% of the marker varied from more than 4 h at pH 5.0 to 64 min at pH 6.0 and 22 min at pH 7.0.

Experimental design

Comparative evaluation of three different breath test substrates for assessment of orocaecal transit time. In order to determine the equivalence of the different breath test substrates for assessment of OCTT, the lactose-13C-ureide, inulin-14C-carboxylic acid and Raftilin HP (hydrogen) breath tests were simultaneously performed in 14 healthy volunteers (mean age 25; seven women and seven men).

In the evening before the test, 1 g of unlabelled lactose ureide was administered in order to induce the appropriate enzyme activity for degradation of lactose-13C-ureide in the colonic bacteria. After an overnight fast, breath samples were taken to determine the basal values of 13CO2, 14CO2 and hydrogen. The substrates for measurement of the OCTT, respectively Raftilin HP (5 g), inulin-14C-carboxylic acid (74 kBq) and lactose-13C-ureide (500 mg) were added to the batter of a pancake test meal which consisted of 8.4 g proteins, 11.2 g fat and 26.7 g carbohydrates (243.5 kcal). After ingestion of the test meal, breath samples were taken at 15-min intervals for 10 h. For 13CO2 and hydrogen measurements, breath was collected in exetainers (PDZ, Cheshire, UK). The 13C breath enrichment was determined by isotope ratio mass spectrometry (PDZ, Cheshire, UK), whereas hydrogen was measured in a H2-monitor (M.E.C., Brussels, Belgium), which contained an electrochemical cell and yielded a digital readout of hydrogen concentration in parts per million (ppm). Breath samples for analysis of 14C were collected by blowing through a pipette into vials containing 2 mmol of hyamine hydroxide until discoloration of the thymolphthalein indicator, corresponding to the capture of 2 mmol of CO2. 14CO2 was measured by β-scintillation counting (Packard Tricarb Liquid Scintillation Spectrometer, model 3375; Packard Instruments Inc., Downers Grove, IL, USA) after addition of 10 mL of Hionic fluoro (Perkin Elmer, Boston, USA). In the lactose-13C-ureide and the inulin-14C-carboxylic acid breath test, calculations were performed directly on measured delta/dpm values. The OCTT was defined as the time at which a significant increase in 13/14C from the background was seen in the breath, i.e. 2.5 times the standard deviation of all previous points above the running average of all previous points. Hydrogen excretion was expressed in ppm. A consistent rise in hydrogen excretion of 10 ppm above baseline was defined as a cut-off value for the OCTT.

Evaluation of the colonic delivery system using the OCTT as a reference. Ten healthy volunteers (mean age 24; five women and five men) participated in the study designed in order to evaluate the in vivo effectiveness of the colonic delivery system. In this study, two tests were performed, respectively before and after a lactulose intake period. During the intake period, the volunteers received for 2 weeks two times per day (once with breakfast, once with supper) 10 g lactulose. No lactulose was taken on the day of the second test.

After an overnight fast, basal breath samples and a basal urine sample were obtained after which the volunteers consumed a pancake test meal containing inulin-14C-carboxylic acid and Raftilin HP, together with the colonic delivery system. Breath samples were obtained and analysed as described above and urine samples were collected in different fractions (0–3, 3–6, 6–9, 9–12 and 12–24 h). All urine was collected in recipients to which neomycin was added for prevention of bacterial growth. After measurement of the volume, samples were taken and stored at −20 °C until analysis.

Determination of urinary Ntot and 15N

Total N content (Ntot) and 15N enrichment were determined by a continuous flow elemental analyser coupled to an isotope ratio mass spectrometer (ANCA-2020; Europa Scientific, Crewe, UK). Therefore, a known amount of urine (15 μL) was absorbed on chromosorb (Elemental Microanalysis Limited, Devon, UK) in a tin capsule, which was introduced in the oxidation-reduction module and combusted to elemental nitrogen. The 15N to 14N isotope ratio of N2 was measured with reference to a calibrated laboratory standard (i.e. a standard ammonium sulphate solution). Also the total N content was measured, based on the nitrogen peak areas, according to the same laboratory standard. Results for 15N were expressed as percentage of the administered dose of 15N recovered in the different urine collections (0–3, 3–6, 6–9, 9–12 and 12–24 h). The percentage of administered dose of 15N recovered was calculated as described previously.

Statistical analysis

Results were expressed as mean and 95% confidence intervals (CIs). The statistical analysis was performed...
with Statistica software (Statistica 6.0; Statsoft Inc. 1984–2001, Tulsa, OK, USA). Statistical evaluation of the data was performed by applying the Student t-test and the analysis of variances (ANOVA), including the Tukey test for differences. Pearson’s correlation coefficient was used to determine the relationship of the OCTTs between the three different breath test substrates.

RESULTS

Comparison of lactose-13C-ureide, inulin-14C-carboxylic acid and Raftilin HP as substrates for assessment of OCTT

The OCTT could be determined in 11 of 14 volunteers using 13CO2-response, in nine of 14 volunteers using 14CO2-response and in 10 of 14 using H2-response. In two volunteers, no response for the three substrates was detected within 10 h. No statistically significant difference was measured in the time at which a significant increase in 13/14CO2 or H2 was seen in breath: the OCTTs were respectively 396 min (95% CI 324–469) for lactose-13C-ureide, 398 min (95% CI 318–478) for inulin-14C-carboxylic acid and 420 min (95% CI 376–463) for Raftilin HP (ANOVA and Tukey test for differences, P = NS) (Figure 1). The OCTT, as determined by the inulin-14C-carboxylic acid breath test, correlated well with the lactose-13C-ureide OCTT breath test (Pearson’s correlation coefficient: r = 0.92, P = 0.003). A moderate correlation was found for the OCTTs obtained between Raftilin HP and inulin-14C-carboxylic acid and between lactose-13C-ureide and Raftilin HP.

Comparison of 13CO2 excretion time, originating from the colonic delivery system, with 14CO2 and H2 excretion time, originating from Raftilin HP and inulin-14C-carboxylic acid as substrates for OCTT assessment

In the tests performed at baseline (i.e. before administration of lactulose), an increase in 13CO2-excretion was observed in eight of 10 volunteers, whereas an increased 14CO2-excretion was found in nine of 10 volunteers and an increased H2-excretion in eight of 10 volunteers. After a 2-week period of lactulose intake, the responses were seven of 10, eight of 10 and six of 10 for respectively 13CO2, 14CO2 and H2. No significant difference was observed between the 13CO2 excretion time [358 min (95% CI 299–416)] and the OCTTs with the inulin-14C-carboxylic acid breath test [376 min (95% CI 321–432)] and Raftilin HP breath test [375 min (95% CI 322–428)]. Correlation of all three methods was highly significant, with r = 0.95 (P = 0.001) for 14CO2 and H2, r = 0.80 (P = 0.03) for 13CO2 and H2 and r = 0.80 (P = 0.03) for 14CO2 and 13CO2.

Upon repetition of the test after a 2-week lactulose intake, the 13CO2 excretion time [383 min (95% CI 320–447)] and OCTTs [354 min (95% CI 304–405) for inulin-14C-carboxylic acid and 392 min (95% CI 342–442) for Raftilin HP] were not significantly different. Again, correlation was highly significant for 13CO2 and H2 with r = 0.88 (P = 0.02) and for 14CO2 and 13CO2 with r = 0.89 (P = 0.02). Also a good correlation was found for 14CO2 and H2 with r = 0.70 (P = NS).

These results are shown in Table 1 and Figure 2. Comparison of the 13/14CO2 and H2 excretion times before and after lactulose intake revealed no statistically significant differences (ANOVA and Tukey test for differences, P = NS).

Urinary 15N excretion

No 15N was found in the 0–3-h urine collection. In both test conditions (i.e. before and after lactulose intake), the appearance of 15N in urine occurred simultaneously with the appearance of 13C in breath. Table 2 summarizes the results of the urinary 15N excretion before and after lactulose intake. No significant differences were found between both test situations. The cumulative excretion of the 15N-isotope after 24 h was not significantly different [56.65% (CI 42.08–71.22) vs. 47.35% (CI 32.77–61.92)] (Figure 3). In three
volunteers, no OCTT was determined and the $^{15}$N enrichment was only observed in the 9–12- or 12–24-h urine collection. In two of the 20 test situations, the first increase in $^{15}$N enrichment was detected in the 12–24-h urine collection.

**DISCUSSION**

In order to use stable isotope labelled substrates for the study of colonic metabolism, the availability of a reliable colonic delivery system, releasing the substrates upon

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**Table 1. Results of the $^{13}$CO$_2$ excretion time (colonic delivery system) and $^{14}$CO$_2$ and H$_2$ excretion time [assessment of orocaecal transit time (OCTT)] before and after lactulose intake**

<table>
<thead>
<tr>
<th>Test substrate</th>
<th>Method</th>
<th>Excretion time (min) Before lactulose intake (95% CI)</th>
<th>Excretion time (min) After lactulose intake (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}$C-urea</td>
<td>Colonic delivery system</td>
<td>358 (299–416)</td>
<td>383 (320–447)</td>
</tr>
<tr>
<td>Inulin-$^{14}$C-carboxylic acid</td>
<td>OCTT</td>
<td>376 (321–432)</td>
<td>354 (303–404)</td>
</tr>
<tr>
<td>Raftilin HP</td>
<td>OCTT P-value</td>
<td>375 (321–428)</td>
<td>392 (342–442)</td>
</tr>
</tbody>
</table>

NS, not significant.

**Figure 2. Simultaneous measurement of $^{13}$C, $^{14}$C and H$_2$ excretion in breath, originating from the colonic delivery system ($^{13}$C-urea) and the Raftilin HP and inulin-$^{14}$C-carboxylic acid breath test for assessment of orocaecal transit time.**

**Table 2. Results of the $^{13}$CO$_2$ excretion time and the urinary $^{15}$N excretion (mean + 95% CI) of the colonic delivery system before and after lactulose intake**

<table>
<thead>
<tr>
<th>Test</th>
<th>$^{13}$C excretion time (min)</th>
<th>Urinary $^{15}$N excretion (% dose $^{15}$N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before lactulose intake</td>
<td>0–3 h 3–6 h 6–9 h 9–12 h 12–24 h</td>
</tr>
<tr>
<td>Before lactulose intake</td>
<td>358 (299–416)</td>
<td>0.03 (−0.01–0.08) 6.97 (0.49–13.45) 13.69 (5.74–21.64) 13.31 (9.23–17.38) 22.64 (16.44–28.84)</td>
</tr>
<tr>
<td>After lactulose intake</td>
<td>383 (320–447)</td>
<td>0.06 (−0.02–0.14) 3.81 (0.21–7.82) 11.12 (2.84–19.39) 8.58 (4.51–15.67) 23.81 (16.60–31.01)</td>
</tr>
</tbody>
</table>

NS, not significant.
arrival in the colon would be of great help. With this application in mind, coating of capsules with a pH-sensitive polymer was selected as a suitable colonic delivery system.

The in vivo performance of the pH-dependent coating used in this study was evaluated by comparing the time at which the substrates incorporated in the capsule were released with the OCTT as determined using two different breath tests.

The substrates incorporated in the colonic delivery system have been chosen in such a way that their release could easily be monitored in breath and urine samples. In the colon, $^{13}$C-urea and $^{15}$N-urea are rapidly hydrolysed to $^{13}$CO$_2$ and NH$_3$ or CO$_2$ and $^{15}$NH$_3$ after which the labelled metabolites are excreted respectively in breath and urine.

The hydrogen breath test using inulin as a substrate has recently been shown to be a reliable test for measurement of OCTT. However, some individuals do not show an increased breath hydrogen excretion after consumption of inulin because of the fact that the hydrogen produced in the colon is metabolized by gut bacteria to methane and hydrogen sulphide rather than be excreted in breath. The prevalence of this so-called ‘hydrogen non-producers’ is estimated at about 25%. Therefore, in order to avoid inconclusive results because of hydrogen non-producers, we preferred to include a second substrate, which was not based on hydrogen excretion, for the estimation of the OCTT. Inulin-$^{14}$C-carboxylic acid was selected as an alternative substrate because, like inulin, it is stable in the upper part of the gastro-intestinal tract whereas upon arrival in the colon, it is metabolized to $^{14}$CO$_2$ which can be measured in breath simultaneously with H$_2$ and $^{13}$CO$_2$. As inulin-$^{14}$C-carboxylic acid had not been validated before as a substrate for OCTT and as the rate of bacterial metabolism of inulin and inulin-$^{14}$C-carboxylic acid is not necessarily the same, a preliminary evaluation was required. The results of this comparison demonstrate that inulin-$^{14}$C-carboxylic acid might serve as an alternative substrate to inulin and lactose-$^{13}$C-ureide in OCTT breath tests. The availability of an additional substrate, labelled with a different isotope, for OCTT measurements can be very useful in some occasions, for instance when several gastro-intestinal parameters have to be evaluated simultaneously. An additional advantage is the fact that the use of inulin-$^{14}$C-carboxylic acid does not require predosing to stimulate bacterial enzyme activity, as is the case with lactose-$^{13}$C-ureide. Furthermore, only very small amounts of the substrate have to be administered [74 kBq corresponds to approximately 1 mg (specific activity: 400 MBq/mmol, M$_r$: 5200)] which implicates that no physiological effects have to be expected. This might be important in studies combining OCTT measurement with evaluation of colonic metabolism. In these cases, inulin cannot be used as inulin, administered in a dose of 5–10 g, influences colonic metabolism because of its prebiotic properties.

On the contrary, although the radiation dose is very low [estimated dose coefficient over 50 years: $\epsilon_{\text{mg}(50)} = 5.8 \times 10^{-10}$ Sv/Bq (ICRP-68, 1994)], the radioactive label precludes its use in children and pregnant women.

In the second part of the study, it was found that the time of appearance of $^{13}$CO$_2$ in breath, originating from released $^{13}$C-urea from the colonic delivery system, correlated very well with the OCTT as measured with both inulin and inulin-$^{14}$C-carboxylic acid, as well as baseline as after a 2-week period of lactulose intake. The measurement of urinary $^{15}$N-excretion enabled us to extend the measurement of degradation of the capsule over a longer time period (24 h instead of 10 h) and to confirm what happened in individuals showing no $^{13}$CO$_2$-response in breath. In two of 10 tests at baseline, no increase in $^{13}$CO$_2$ excretion was observed. In one of these persons, no increased excretion was found neither for $^{14}$CO$_2$, H$_2$ nor $^{13}$CO$_2$, suggesting that this person had an OCTT longer than 600 min. Indeed, the first increase in urinary $^{15}$N-enrichment was found in the 9–12-h urine collection. In the second person, the OCTT was found to be 450 min whereas the first increase in $^{15}$N-enrichment was only detected in the 12–24-h urine collection indicating a delayed release of the colonic delivery system.

As we used a pH-dependent coating, the study was repeated after a 2-week intake period of a non-digestible

Figure 3. Cumulative per cent dose-$^{15}$N excretion ($\pm$ s.d.) before and after lactulose intake in the different urine fractions.
fermentable carbohydrate, i.e. lactulose. It is well known that upon arrival in the colon, these carbohydrates are fermented by the bacterial flora with production of short chain fatty acids, resulting in an acidification of the colonic contents. In this way, we tried to mimic the colonic environment of people who are used to consume a diet rich in carbohydrates and fibres. It was anticipated that in these individuals, the degradation of the system could be delayed or could even fail to occur. However, as the degradation of inulin and inulin-14C-carboxylic acid is caused by bacterial enzymes and is not impaired at a lower pH, and as no difference was observed between the time of appearance of 13CO2 and 14CO2 or H2, it was concluded that the acidification caused by the diet intervention with lactulose did not interfere with the degradation of the colonic delivery capsule.

After the lactulose intake period, no increase in 13CO2 excretion was found in three of 10 tests. Two individuals seemed to have an OCTT longer than 600 min (no signal in breath with either of the substrates) and one person had a OCTT of 300 min but a delayed release of the colonic delivery system (15N-excretion only in the 12–24-h collection). As a consequence, in none of the volunteers, the contents of the colonic delivery system have been released before arrival in the colon and only in two of 20 individuals, the contents of the coated capsule has been released at a significantly later time point than the OCTT.

An even more significant correlation was found between the time of appearance of 13CO2 in breath and the OCTT, when the results of the individuals in which an OCTT was determined but had a delayed release of the colonic delivery system, were not taken into account. At baseline the correlation between 13CO2 and H2 was 0.80 (P = 0.03) and for 13CO2 and 14CO2 the correlation was 0.90 (P = 0.002). After the 2-week lactulose intake period, the correlations became r = 0.96 for 13CO2 and H2 (P = 0.01) and r = 0.88 for 13CO2 and 14CO2 (P = 0.008). Also a highly significant correlation was found when comparing the results obtained for the OCTTs of inulin and inulin-14C-carboxylic acid of both test conditions with r = 0.70 (P = 0.00034) as shown in Figure 4.

Figure 4. Correlation between the simultaneously performed inulin- and inulin-14C-carboxylic acid breath tests (OCTT, oro-caecal transit time).

These results indicate that the colonic delivery system used in this study seems a very promising tool to deliver labelled substrates to the colon and might be used in metabolic studies. In addition, the fact that the degradation of the capsule starts quasi immediately upon arrival in the colon opens perspectives to use the present formulation (polymer coated capsule containing 13C-labelled urea) as another alternative substrate for estimating OCTT. For instance, in patients who have recently received an antibiotic treatment, the OCTT cannot be determined using the currently available substrates which are all based on bacterial degradation. In these patients, the current capsule could be useful as its degradation does not depend on bacterial activity. In addition, it could be investigated whether the capsule could be useful for measurement of OCTT in patients with small bowel bacterial overgrowth. In these patients, the standard substrates for OCTT measurement (inulin, inulin-14C-carboxylic acid or lactose-13C-ureide) are likely to be metabolized before arrival in the colon which will result in too short estimates of OCTT. Provided that the increase in luminal pH, often accompanying small bowel bacterial overgrowth, is limited, the capsule might provide more reliable values. However, as mentioned before, this remains to be investigated.

In conclusion, the results in this study have shown that the performance of this pH-sensitive colonic delivery system could be demonstrated using isotope techniques and that such a system seems very attractive to deliver labelled substrates to the colon. Furthermore, inulin-14C-carboxylic acid has been shown to be a suitable alternative substrate for the assessment of OCTT, especially in situations where several gastrointestinal parameters have to be studied simultaneously.
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REFERENCES

11 Levine D.S, Raisys V.A, Aimardi V. Coating of oral beclo- methasone dipropionate capsules with cellulose acetate phthalate enhances delivery of topically active anti-inflammatory drugs to the terminal ileum. Gastroenterology 1987; 92: 1037–44.