Gastric emptying flow curves separated from carbon-labeled octanoic acid breath test results

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RECENTLY, WE DEVELOPED the [13/14C]octanoic acid breath test to measure gastric emptying of solids. Although the method has been validated extensively, absorption, metabolism, and excretion of the label in the breath need to be corrected for. In this study a mathematical model was developed that allows for 1) separation of the global CO2 excretion after ingestion of the labeled test meal into the emptying rate of the labeled test meal from mouth to pylorus and the postgastric processing of absorption, metabolism, and excretion of the label, and 2) numerical calculation of the half-emptying time and lag phase of the emptied meal. The model was applied to the gastric emptying results obtained by simultaneous scintigraphic and breath test measurements. An excellent correlation was found between the radioisotopic and breath test results. An excellent correlation was found between the two methods [mean values and confidence limits for differences: tr = 10 min (−20 to 41) and tlag = −3 min (−39 to 34)]. Moreover, the separated gastric emptying curves, lacking the influence of postgastric processing of the label, showed real patterns of gastric outflow, which changes from moment to moment.

breath test technology; mathematical models

Recently, we developed the [13/14C]octanoic acid breath test to measure gastric emptying of solids (7, 16). The rationale of a breath test is based on the firm retention of 13/14C-labeled octanoic acid in the solid phase of a test meal to obtain real-time gastric emptying curves. This approach of breath test curve analysis has two potential benefits: 1) physiologically meaningful gastric emptying parameters can be calculated from breath test curves without correcting for postgastric processing of the label on a linear regression-estimated basis between scintigraphic and breath tests, and 2) it allows for the evaluation of gastric emptying rates, instead of amounts of emptied food, as a function of time (flow curves). The classic multicompartmental analysis, however, was not used due to the specific conditions encountered in breath test technology. The multiple-chamber model is difficult to apply in clinical practice, because the dynamic exchange of CO2 with the rapidly and slowly exchanging compartments cannot rarely be done convincingly with biological data, even if sampling takes place over long periods of time. Also, it is not possible to obtain a steady state of exchange between the different compartments (especially the slowly exchanging ones) during the 4-h period of breath sampling. Moreover, when dose is not in the steady state, the rate constants for intercompartmental exchange cannot be explicitly calculated from the multieponential function for tracer in breath.

RATIONALE FOR THE SEPARATION MODEL

To elaborate the mathematical model, three functions were introduced to describe three different processes.

1) The emptying rate of a labeled solid meal from mouth to pylorus is given by M(t).

2) The rate of postgastric processing (absorption, metabolism, and excretion in breath) of the label is given by D(t).

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The global process of CO₂ excretion after ingestion of a labeled solid test meal is given by T(t).

The aim of this study is to determine M(t) given T(t) and D(t), which can both be measured, and to describe the relation between the three functions. Therefore the following assumptions were made. 1) The meal is ingested at once, at time 0. This is not true, but the time of ingestion was always restricted to 10 min, and time 0 was taken as the time of completion of the ingestion of the meal. 2) T(t), D(t), and M(t) are piecewise continuous functions, not identical to zero and positive for each time \( t = 0 \). 3) The rate of metabolism of the label \( D(t) \) is proportional to the rate of gastric emptying of the label \( M(t) \). This implies that the kinetics of metabolism of the label are independent of the rate at which the label is emptied [no saturation of \( D(t) \) as a function of \( M(t) \)], or, stated differently, that \( D(t) \) is invariant of \( M(t) \).

We first demonstrate that, in theory, under the assumptions made above, the separation model is a mathematically correct alternative to the multicompartamental model to separate a function (i.e., gastric emptying rate) from a global process when rate constants for intercompartmental exchange cannot be explicitly calculated. We then demonstrate the practical elaboration of deriving the gastric emptying rate from labeled octanoic acid breath test curves and the proportionality of \( D(t) \) to \( M(t) \).

**DESIGN OF THE SEPARATION MODEL**

To simplify the rationale of the model, \( T(t) \), \( D(t) \), and \( M(t) \) are not considered to be continuous but are divided into discrete time intervals. The rate of \(^{13/14}\text{CO}_2\) excretion during a certain time interval is the result of the accumulated effect of parts that have left the stomach in the past intervals (Fig. 1). For example, the rate of the part of the label that left the stomach in the first time interval but was metabolized during the second time interval (simplified: the first passage in the liver), the part of the label that left the stomach in the second time interval but had already been metabolized during the first time interval (simplified: the first passage in the liver). The addition of all layers describes the total process \( T(t) \), i.e., a \(^{13/14}\text{CO}_2\) excretion curve after ingestion of a solid test meal. Mathematically it is expressed as

\[
T_2 = M_1D_1 \\
T_3 = M_1D_2 + M_2D_1 \\
T_4 = M_1D_3 + M_2D_2 + M_3D_1 \\
T_5 = M_1D_4 + M_2D_3 + M_3D_2 + M_4D_1 \\
or, in general, as
\]

\[
T_n = \sum_{i=1}^{n-1} D_{n-i}M_i
\]

By decreasing the length of the time intervals to zero, the formula becomes a continuous function

\[
T(t) = \int_0^t D(t - t_0)M(t_0) dt_0
\]

The relationship between the different rates as described in equation 2 is mathematically known as a convolution product. A number of properties can easily be derived mathematically. However, these properties are not of interest in this study, since it is not possible in general to find the inverse relation between \( T(t) \) and \( M(t) \), except for special classes of functions such as \( e^t \). Such functions are used in Fourier and in Laplace transforms, but these functions do not have the form observed in our data. Therefore, we have used the discrete formalism (Eq. 1) to derive a discrete calculation in practice

\[
M_1 = \frac{T_2}{D_1} \\
M_2 = \frac{T_3 - D_3M_1}{D_1} \\
M_3 = \frac{T_4 - D_3M_1 - D_2M_2}{D_1} \\
or, in general
\]

\[
M_i = \frac{T_{i+1} - \sum_{j=1}^{i-1} D_{i+1-j}M_j}{D_1}
\]

If \( T(t) \) and \( D(t) \) are known, \( M(t) \) can be separated from the total process \( T(t) \) by decreasing the length of the time intervals.

**ELABORATION OF THE MODEL**

**Methods**

Subjects and materials. As functions of \( T(t) \), the \(^{14}\text{CO}_2\) excretion data obtained in the validation study comparing the \(^{14}\text{C}\)octanoic acid breath test and the radioscintigraphic technique were used (7). Briefly, in
this study a standard solid test meal (250 kcal) consisting of one egg (labeled with 74 kBq of \[^{14}C\]octanoic acid and 110 MBq of \[^{99m}Tc\]-labeled albumin colloid), two slices of bread, and 5 g of margarine was ingested by 16 healthy volunteers and 20 dyspeptic patients. Immediately after ingestion of the meal, each subject was seated between the two heads of a dual-headed gamma camera equipped with parallel-hole low-energy collimators and interfaced to a computer. Scanning scintigraphic information was obtained every 10 min for up to 1 h and every 15 min for another period of 1 h. Radioactivity remaining in the stomach at each scanning period was expressed as a percentage of the activity initially present. The gastric emptying rate so obtained was fitted by the modified power exponential formula of Siegel et al. (24). The half-emptying time obtained was fitted by the modified power exponential activity initially present. The gastric emptying rate so

The mean \({14}CO_2\) excretion curve obtained in 20 healthy volunteers after intraduodenal administration of 74 kBq of \[^{14}C\]octanoic acid served as the function \(T(t)\). As far as the function \(M(t)\) is concerned, no class of functions exists. Accurate fitting of this curve is done by a combination of exponential and polynomial functions.

I. Ascending slope: \(c(1 - e^{-at^b})\)

II. Descending slope: \(e^{-at^{b+g}}\)

III. Binding of I and II: \(h + it + jt^2 + kt^3 + lt^4\)

where \(t\) is time and \(a, b, c, d, f, g, h, i, j, k, \) and \(l\) are regression-estimated constants.

Using these equations for \(T(t)\) and \(D(t)\), in Eq. 3 the curve \(M(t)\) is obtained. Two gastric emptying parameters were calculated numerically from the individual curves \(M(t)\): 1) the gastric half-emptying time is calculated by solving the equation

\[
\int_{0}^{t_{1/2}} M(t) \, dt = \frac{1}{2} \int_{0}^{\infty} M(t) \, dt
\]

and 2) the lag phase \(t_{lag}\), as defined by Siegel et al. (24), which corresponds to the time of peak excretion in the function \(M(t)\). Statistics. The gastric half-emptying times and lag phases of the separated functions of \(M(t)\) were calculated numerically after integration into \(M(t)\) as a function of time and were compared with the scintigraphically determined half-emptying times and lag.
phases of the validation study (7), using correlation analysis [SAS: PROC CORR (21)]. The two tests were further compared using the Bland and Altman procedure (3). The three parameters for evaluation of the kinetics of metabolism of [14C]octanoic acid after intraduodenal administration were compared for the three boluses using the Mann-Whitney-Wilcoxon test (21).

RESULTS

Postgastric Processing of [14C]Octanoic Acid

Figure 2 represents 14CO2 appearance in breath after intraduodenal administration of 74 kBq of [14C]octanoic acid in the second part of the duodenum in 20 healthy volunteers (means ± SE).

Fig. 2. Dynamics of 14CO2 appearance in breath after intraduodenal administration of 74 kBq of [14C]octanoic acid in the second part of the duodenum in 20 healthy volunteers (means ± SE).

Invariance of D(t) from M(t)

In Fig. 3, the 14CO2 excretion as a function of time is given in six subjects, after intraduodenal administration of three different boluses of [14C]octanoic acid. At each bolus injection of [14C]octanoic acid, peak excretion in breath was reached at 10 ± 0.83 min, with a peak of 33.05 ± 2.49% dose/h after the first bolus, 24.18 ± 1.54% dose/h after the second bolus, and 28.61 ± 2.03% dose/h after the third bolus. The increase in 14CO2 excretion 10 min after injection of the bolus was 33.05% (0.447% per injected kBq of activity) at 10 min, 8.18% (0.442%/kBq) at 70 min, and 16.59% (0.448%/kBq) at 130 min. The area under the curve during the first hour was 22.99 ± 1.20% (0.31% per injected kBq of activity) for the first injected bolus of 74 kBq, 7.17 ± 0.47% (0.39%/kBq) for the second bolus of 18.5 kBq, and 10.64 ± 0.54% (0.29%/kBq) for the third bolus of 37 kBq. The differences between the three boluses for the three parameters (parameters 2 and 3 calculated per kBq of activity) were statistically not significant.

Application of the Model

Figure 4 depicts the relationship between the three functions T(t), M(t), and D(t) in two subjects after ingestion of a [14C]octanoic acid-labeled standard solid test meal. The first subject had a normal gastric emptying rate with a scintigraphically determined half-emptying time of 59 min (Fig. 4A): the rate of gastric emptying accelerates very quickly before reaching a peak, followed by a gradual decline in the velocity of gastric emptying. The second subject had a delayed gastric emptying rate with a scintigraphically determined half-emptying time of 89 min (Fig. 4B); the acceleration and deceleration in the gastric emptying rate is less pronounced and less steep. By analyzing the gastric emptying data in this way, it was clear that gastric emptying velocity changes from minute to minute and never has a constant value.

The separated gastric emptying function M(t) allowed not only for evaluation of the real pattern of emptying but also for the calculation of a half-emptying time. The relationship between the gastric half-emptying times determined scintigraphically and via breath test in 16 healthy volunteers and 20 dyspeptic patients after ingestion of a dually labeled solid test meal of 250 kcal is given in Fig. 5A. The correlation coefficient between the two parameters was 0.98. Figure 5B gives the relationship between the lag phases obtained by both techniques, defined as the point of maximal gastric emptying rate according to the method of Siegel et al. (24). The correlation coefficient was 0.85. The Bland and Altman plots of gastric half-emptying times and lag phases determined scintigraphically and via breath test, given in Fig. 6, showed, first, an offset between both methods not significantly different from zero, and second, no proportional differences between the two methods [mean and confidence limits for differences between the methods: t1/2, 10 min (–20 to 41) and tlag, –3 min (–39 to 34)].

DISCUSSION

This study aimed to develop a mathematical model to separate one physiological function from breath test analysers.
results. All breath tests are based on the administration of a substrate with a functional group containing a carbon atom with either the radioactive ($^{14}$C) or the stable ($^{13}$C) isotope of carbon. The functional group is enzymatically cleaved during passage through the gastrointestinal tract, during its absorption, or in subsequent metabolic processes. After cleavage of the target bond, the cleaved portion undergoes further metabolism to $^{14}$CO$_2$ or $^{13}$CO$_2$, which mixes with the bicarbonate pool of blood and is finally expired in the breath. In this way, $^{14/13}$CO$_2$ excretion is a reflection of the total amount or kinetic properties of the enzyme studied, given that this enzyme relates to the rate-limiting step in the whole process.

By applying this mathematical model to the [$^{13/14}$C]octanoic acid breath test to measure gastric emptying of solids, we were able to demonstrate that postgastric processing of [$^{13/14}$C]octanoic acid until $^{13/14}$CO$_2$ exhalation occurs very rapidly, with minimal intersubject variability. This is due to very rapid absorption from the small intestine, quick transport to the liver [no mucosal esterification, no incorporation in chylomicrons (10, 18–19)], and a ready and almost complete oxidation to $^{13/14}$CO$_2$ in the liver [no requirement for carnitine to cross the double mitochondrial membrane (4, 22)]. Therefore, gastric emptying of the meal can be considered the rate-limiting step in $^{13/14}$CO$_2$ excretion after ingestion of a [$^{13/14}$C]octanoic acid-
labeled solid meal. Also, an average function can be used to describe the "postgastric processing" of octanoic acid. Metabolism of octanoic acid remains unaltered not only in healthy volunteers but also in other circumstances, as has been shown for insulin-dependent diabetes mellitus (14) or after administration of octreotide (15).

The assumption of invariance of postgastric processing of [13/14C]octanoic acid from the rate of emptying from the stomach was fulfilled in this study. Hence all other assumptions made were also fulfilled and the separation model could be applied by "subtracting" the shape of the postgastric processing curve on each moment from the global 13/14CO2 excretion curves after ingestion of a labeled meal, in a continuous way and according to the amount of label that has left the stomach at that moment.

The results obtained with the separation model are excellent. The model allows gastric half-emptying time and lag phase to be calculated very accurately and it also provides a method to evaluate patterns of gastric emptying velocity or flow, which changes from minute to minute. In 1990, Schulze-Delrieu (23) pointed out that radioscintigraphic gastric emptying results, expressed as a percentage of the initial amount still remaining in the stomach, represent cumulative data (i.e., mathematical integration of a velocity curve, or "distance" rather than "velocity") and that "gastric emptying rates determined in this way do not allow any conclusions regarding the rate or pattern of actual gastric outflow and identical emptying rates may hide major differences in flow pattern." A gastric emptying flow curve can be obtained from radioscintigraphic data by taking the first derivative of the measured curve. However, mathematical derivation is less stable than mathematical integration. This leads to inaccuracies for calculation of kinetic parameters such as the lag phase, as defined by Siegel (24), since it is mathematically easier to determine the peak of a flow curve than to determine the point of inflection of a cumulative curve. This could be the explanation for a less good correlation of the lag phases of both techniques in this study.

On the other hand, the separation model has its limits. By using fitting curves for the actual measured data of 13/14CO2 excretion, the transpyloric flow is smoothed to a general flow curve and does not display the gushes of chyme leaving the stomach in a pulsatile way.

The separation model presented has a theoretical advantage compared with the classical multiple chamber model (8), in that it makes fewer assumptions. It makes no assumptions about laws governing the flow stream of the label. Moreover, the multiple chamber model is difficult to apply in clinical practice, as discussed in the introduction. The use of the curve D(t), representing the postgastric processing of the label, in separating M(t) out of T(t) and D(t) is an appropriate solution to these problems because D(t) is shown to be proportional to M(t).

In conclusion, an accurate mathematical model was developed to separate gastric emptying flow curves...
from $^{13/14}$CO$_2$ excretion curves obtained after ingestion of a $^{13}$C-octanoic acid-labeled solid test meal, thereby also excluding the influence of endogenous CO$_2$ production on breath test results. The model also has attractive prospects for other (breath) tests to separate a specific gastrointestinal function, e.g., separation of the process of intraluminal lipolysis out of the data of a mixed triglyceride breath test and separation of the assimilation of carbohydrates from gastric emptying of the given test meal.

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