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

B. D. Maes, G. Mys, B. J. Geypens, P. Evenepoel, Y. F. Ghoos, and P. J. Rutgeerts

Maes, B. D., G. Mys, B. J. Geypens, P. Evenepoel, Y. F. Ghoos, and P. J. Rutgeerts. Gastric emptying flow curves separated from carbon-labeled octanoic acid breath test results. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G169–G175, 1998.—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 the evaluation of gastric emptying rates, and the half-emptying time and lag phase, correcting for the postgastric processing of octanoic acid.

The aim of this study was to develop a separation model in which the postgastric processing of octanoic acid could be mathematically separated from the CO2 excretion curve after ingestion of a standard solid 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 rapid and slow bicarbonate pool and the loss of label via excretion in urine and feces and incorporation into bone is difficult to estimate in humans, certainly in each individual. Solution of the breath curve would require the fitting of at least four exponential functions. This can 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 subsequently measured compartment the rate constants for intercompartmental exchange cannot be explicitly calculated from the multieponential curve 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).
3) 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. 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 multicompart-mental 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 13/14CO₂ 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 accumulated effect of parts that have left the stomach during a certain time interval is the result of the 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

\[
T(t) = \int_{t_0}^{t} D(t - t_0)M(t_0) dt_0
\]

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 and M, except for special classes of functions such as e^x. Such functions are used in Fourier and in Laplace trans-forms, 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

\[
M_1 = \frac{T_2}{D_1}
\]

\[
M_2 = \frac{T_3 - D_2M_1}{D_1}
\]

\[
M_3 = \frac{T_4 - D_2M_1 - D_2M_2}{D_1}
\]

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 14CO₂ excretion data obtained in the validation study comparing the [¹⁴C]octanoic acid breath test and the radioscintigraphic technique were used (7). Briefly, in
in this study a standard solid test meal (250 kcal) consisting of one egg (labeled with 74 kBq of [14C]octanoic acid and 110 MBq of 99mTc-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 ($t_{1/2}$) and lag phase ($t_{lag}$) were calculated according to that formula. Breath sampling for 14CO2 followed the same schedule as the scintigraphic imaging technique but continued for another 2 h of sampling, during which breath was collected and measured in 15-min intervals. The results were expressed as the percentage of 14C recovery per hour and were further analyzed by nonlinear regression analysis to calculate $t_{1/2}$ and $t_{lag}$. The gastric emptying parameters of both techniques were compared by correlation and linear regression analysis in this study.

To obtain the function $D(t)$, 20 healthy subjects (10 women and 10 men, mean age 23 yr, range 18–28 yr) were examined. None of the subjects had a history of gastrointestinal disease or surgery and none were taking medication. After an overnight fast, a flexible tube was positioned in the second part of the duodenum under radioscopic control. The dynamics of 14CO2 appearance in breath were measured after intraduodenal administration of 74 kBq of [14C]octanoic acid sodium salt (DuPont NEN, Boston, MA), dissolved in 20 ml of water. Breath samples were taken before and every 3 min during the first 30 min, every 5 min for the next 30 min, and every 15 min thereafter for up to 4 h. The 14CO2 excretion curves were evaluated by 1) the 14CO2 peak excretion time, 2) the 14CO2 peak excretion, and 3) the half-emptying time of the curve (using the formula for $D(t)$).

To validate the invariance of $D(t)$ from $M(t)$, six healthy volunteers (3 women, 3 men; age 18–24 yr) were studied. None of the subjects had a history of gastrointestinal disease or surgery and none were taking medication. After an overnight fast, a flexible tube was positioned in the second part of the duodenum under radioscopic control, and 129.5 kBq of [14C]octanoic acid sodium salt (DuPont NEN, Boston, MA), dissolved in 50 ml of water was injected into the second part of the duodenum in a bolus at three different times: 74 kBq at time 0, 18.5 kBq (1/4 of the initial dose) 1 h later, and 37 kBq (1/2 of the initial dose) at 2 h. Breath samples were taken every 5 min for 4 h. The kinetics of metabolism of each bolus of [14C]octanoic acid were evaluated by three parameters: 1) the time until peak excretion of 14CO2 in breath, 2) the maximal increase of 14CO2 excretion after injection of each bolus, and 3) the increase in area under the curve of 14CO2 in breath obtained during the first hour after injection of each bolus of [14C]octanoic acid (using the formula for $D(t)$).

The study protocol was approved by the ethics committee of the University of Leuven. Informed consent was obtained from all subjects.

Measuring techniques and mathematics. 14CO2 in breath was collected by blowing through a pipette into vials containing 2 ml of 1 M hyamine hydroxide and 2 ml of ethanol together with one droop of thymolphthalein solution. This amount of hyamine is neutralized by 2 mM of CO2. The end point of neutralization is indicated by decoloration of the indicator. After decoloration, 10 ml of scintillation cocktail (Hionic Fluor, Packard Instruments) were added and radioactivity was determined by liquid scintillation spectrometry (Packard Tri-Carb liquid scintillation spectrometer, model 3375; Packard Instruments, Downers Grove, IL). CO2 production was assumed to be 300 mmol per square meter of body surface per hour. Body surface area was calculated by the weight-height formula of Haycock et al. (9). The results were expressed as the percentage of 14C recovery per hour as a function of time.

Application of the Model

The function $T(t)$ can be adequately described in both healthy volunteers and subjects with abnormal gastric emptying rates (1) by two classes of function: $at^{b}e^{-kt}$ or $mkb^{-kt}(1-e^{-kt})^{-1}$, where $t$ is time and $a$, $b$, $c$, $m$, $k$, and $β$ are regression-estimated constants.

The mean 14CO2 excretion curve obtained in 20 healthy volunteers after intraduodenal administration of 74 kBq of [14C]octanoic acid served as the function $D(t)$. As far as the function $D(t)$ 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: $e^{-at^{b}+c}$

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

$$\int_{0}^{t_{1/2}} M(t) dt = \frac{1}{2} \int_{0}^{t} M(t) dt$$

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 excretion as a function of time in 20 healthy subjects, after intraduodenal administration of 74 kBq of [14C]octanoic acid (means ± SE). 14CO2 appeared in the breath almost immediately, with a peak excretion of 33.73 ± 1.69% dose/h after 10.69 ± 0.77 min, followed by an exponential decrease of 14CO2 activity in the breath. The half-excretion time of the curves was 67.5 ± 1.37 min.

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 74, 18.5, and 37 kBq of [14C]octanoic acid in the second part of the duodenum at 0, 1, and 2 h, respectively, in 6 healthy volunteers (means ± SE).

Figure 3. 14CO2 appearance in breath after intraduodenal administration of 74, 18.5, and 37 kBq of [14C]octanoic acid in the second part of the duodenum at 0, 1, and 2 h, respectively, in 6 healthy volunteers (means ± SE).
results. All breath tests are based on the administration of a substrate containing a functional group with a carbon atom with either the radioactive (14C) or stable (13C) 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 14CO2 or 13CO2, which mixes with the bicarbonate pool of blood and is finally expired in the breath. In this way, 14/13CO2 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 [13/14C]octanoic acid breath test to measure gastric emptying of solids, we were able to demonstrate that postgastric processing of [13/14C]octanoic acid until 13/14CO2 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 13/14CO2 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 13/14CO2 excretion after ingestion of a [13/14C]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 13CO2 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 $^{13/14}$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.

Address for reprint requests: P. J. Rutgeerts, Dept. of Medicine and Medical Research, University Hospital Gasthuisberg, B-3000 Leuven, Belgium.

Received 16 December 1996; accepted in final form 4 March 1998.

REFERENCES