Techniques

Determination of total gastric volume, gastric secretion and residual meal using the double sampling technique of George

E J BECKERS, N J REHRER, F BROUNS, F TEN HOOR, AND W H M SARIS

From the Department of Human Biology, University of Limburg, Maastricht, The Netherlands

SUMMARY In literature several techniques are described for the measurement of gastric emptying. One of these is the double sampling technique of George which has the advantage that it enables multiple measurements of total gastric volume in one experiment. In order to estimate net gastric emptying, however, it is important to differentiate between the test meal volume present in the stomach and gastric secretion.

In the present study George’s technique was extended with a calculation procedure to estimate gastric secretion and net test meal outflow. An in vitro experiment was carried out which showed an accuracy of 5–10% for this method. An in vivo comparison was made with Hunt’s chloride technique, which is used for estimating gastric secretion. Sixteen subjects participated in a 60 minute test with water as a test meal. Regression analysis showed a high degree of correlation (R²=0.99) between Hunt’s method and the proposed method. The data obtained indicate that the proposed method gives a reliable estimation of net gastric emptying and is simple to do as no further measurements have to be carried out other than those needed for George’s method.

A suitable and widely used technique to measure gastric volume is the double sampling technique described by George. This method depends on the determination of dye concentration in the gastric samples obtained before and after adding a known amount of dye to the stomach contents. Determinations can be repeated as often as needed and each determination is independent. The assumptions made in George’s method are that during the procedure complete mixing of dye and gastric contents is obtained, the amount of dye absorbed or secreted by the stomach during the sampling period is negligible, no water is absorbed by the stomach, and gastric emptying does not occur during mixing or sampling or can be corrected for. Correction for the latter might prove difficult, however, and can introduce new inaccuracies as gastric emptying is not a linear process.

A disadvantage of George’s method is that it only gives the possibility of measuring the total volume of gastric contents, which in fact consist of gastric secretion, swallowed saliva, and consumed meal. In most experiments, however, it is interesting to differentiate between the volumes of a test meal in the stomach and of gastric juice secreted over a certain period. Hunt proposed a solution for this problem by measuring not only the concentration of the marker added according to George’s method, but also the chloride concentration in the gastric contents. He stated that if the chloride concentration of the gastric juice is constant, the volume of gastric secretion can be calculated and thus also the volume of the test meal. A critical point in Hunt’s method is the assumption that the chloride concentration in secreted gastric juice is constant over a longer period and the same for all subjects. Nordgren and

Address for correspondence: Dr E J Beckers. Department of Human Biology, University of Limburg, PO Box 616, 6200 MD Maastricht, The Netherlands. Received for publication 21 June 1988.
Hobsley\(^4\) showed that chloride concentration depends on the excretion rate of gastric juice. If the secretion rate rises, chloride concentration rises as well. Furthermore, several authors showed\(^5\)-\(^12\) that there is a marked difference in chloride concentration in the gastric secretion between subjects.

In fact there are two rather simple methods to differentiate between meal and gastric juice. One of these methods is adding a second non-absorbable marker to the meal at the start of the experiment. By using one marker for the determination of the total volume (George’s method\(')\) one can calculate the amount of test meal left, based on the dilution of the second marker. This method needs extra measurements which increases the chance of errors. Another disadvantage is that at the end of the experiment the concentration of the second marker might become very low and difficult to measure, because of dilution with gastric secretion. The second is George’s extended with a calculation as described below. In this way the total volume of gastric contents and the volume of a liquid test meal can be measured without the need of extra experiments or measurements.

**Calculation**

The abbreviations used are explained as follows: capital letters represent a variable, \(V\) for volume, \(C\) for concentration, and \(A\) for amount. Raised suffixes are \(s\) for gastric contents, \(i\) for dye, \(t\) for test meal. Lower suffixes are \(n\) for the serial number of the observation, \(b\) for before, and \(a\) for after adding the dye. (For a complete list of variables and equations see the addendum.) The theory runs as follows: let the volume of gastric contents at determination number \(n\) before adding dye be \(V_{nb}(ml)\) and the dye concentration in the stomach at that moment \(C_{nb}(mg/ml)\). After adding a volume \(V'_{n}(ml)\) of dye with concentration \(C'_n(mg/ml)\), the volume of the gastric contents \((V'_{nb})\) will be \(V_{nb} + V'_{n}\) and the dye concentration in the stomach at that moment \(C'_{na}\). The absolute amount of dye present in the stomach after adding dye is equal to the amount of dye present before adding dye plus the amount of dye added. This results in the equations given by George\(')\ (addendum eq 1 and eq 2). If a test is done it will start by giving a test meal with a certain dye concentration, thus in the first determination of a series the volume of dye added \((V'_{1})\) will be the volume of the test meal given \((V_{1})\) and \(C_{1}\) its dye concentration, while \(C_{nb}=0\). In all following determinations \(V'_{n}\) is the volume and \(C'_{n}\) the concentration of the dye solution added to the gastric contents during the determination. Using the same data as obtained for the first determination of the volume of gastric contents according to George’s method, calculation of the amount of test meal left in the stomach at the \(n\)-th determination can be done as follows: after the addition of dye solution in the first determination of the volume of gastric contents, the amount of dye in milligrams in the stomach \((A_{1a})\) equals the concentration of dye in the stomach after adding dye multiplied by the volume of the gastric contents at that time (addendum eq 3). \(A_{1a}\) is equal to the amount added to the test meal and is thus representative for the volume of the test meal \((V'_{1})\). In the second determination the amount of dye in the stomach before adding dye solution is \(A_{2b}\) milligrams (addendum eq 4). The ratio of \(A_{2b}\) over \(A_{1a}\) represents the fraction of \(A_{1a}\) still present in the stomach at the second determination. As \(A_{1a}\) is equal to the amount of dye in the test meal, the volume of the test meal which is still present in the stomach at the second determination of gastric contents, \(V'_{2}\), can be calculated from this ratio of \(A_{2b}\) over \(A_{1a}\) (addendum eq 5). Similarly the ratio of \(A_{3b}\) over \(A_{2a}\) represents the fraction of \(A_{2a}\) still present in the stomach at the third determination of the volume of gastric contents. As \(A_{2a}\) is equal to the amount of dye in the stomach after adding dye in the second determination, while the volume of test meal present in these contents is \(V'_{2}\), the volume of the test meal still present in the stomach at the third determination \((V'_{3})\) can be calculated by combining these factors (addendum eq 6). This yields the following general formula:

\[
V'_{n} = \frac{A_{nb}}{A_{(n-1)a}} \cdot \frac{A_{(n-1)b}}{A_{(n-2)a}} \cdot \frac{A_{2b}}{A_{1a}} \cdot \frac{V'_{1}}{V'_{2}}
\]

(eq 8)

Where \(V'_{n}(ml)\) is the volume of the test meal still present in the stomach at the \(n\)-th determination of the volume of gastric contents and \(A\) (mg) is the amount of dye present in the stomach at a certain moment. The indices \(a\) and \(b\) denote after and before adding dye respectively and \(V'_{1}(ml)\) is the volume of the original test meal.

It can be seen from equation 1 that the determination of the volume of gastric contents \(V'_{nb}\) using the method of George is fully independent of former determinations. It only depends upon the added volume of the dye solution \((V'_{nb})\), the concentration of this solution \((C'_{nb})\) and the dye concentration in the stomach measured before \((C'_{nb})\) and after \((C'_{na})\) adding the dye respectively. On the contrary, calculation of the volume of the test meal still present in the stomach at the \(n\)-th determination of the volume of the gastric contents using equation 3, is fully dependent upon all determinations of \(V\) and \(C\) made before. Thus one wrong determination will influence all the following ones.
Determina­tion of total gastric volume, gastric se­cre­tion and res­i­dual meal using the tech­nique of George

Methods

To check this method an in vitro ex­per­i­ment was per­formed. A test meal of 600 ml water (V₁ = Vₐ₁) with a mark­er (phenol red) con­cen­tra­tion of 15 mg/l (C₁ = Cₙ₁) was used. A start­ing vol­ume of the stom­ach of 50 ml (Vₐ₂ₙ) gas­tric juice pre­sent in the stom­ach at the on­set of the test, an em­pty­ing rate of 100 ml and a pro­duc­tion rate of 20 ml gas­tric juice per in­ter­val were as­sumed, re­sult­ing in a net em­pty­ing rate of 80 ml per in­ter­val. Tₐ and Tₙ are the in­ter­val times be­fore and after add­ing 5 ml con­cen­trated phenol red (500 mg/l) as a mark­er. Mix­ing was per­formed by aspir­ing and rein­ject­ing the ‘stom­ach con­tents’ with a 50 ml syringe (mo­noject, catheter­tip, nr 1100–560281) for one min­ute, and dur­ing this pe­ri­od a pump­ing ac­tion was per­formed at least 12 times. Sam­ple tak­ing, add­ing of ‘stom­ach juice’ and with­draw­ing ‘gas­tric con­tents’ was per­formed with sim­i­lar syringes. Phenol red mark­er was added using a 10 ml Methrom piston burette. NaCl was added to the or­igi­nal test meal in a con­cen­tra­tion of 150 mmol/l as a sec­ond mark­er. As there will not be any fur­ther add­ing of NaCl, the am­ount of liq­uid test meal left in the stom­ach can be cal­cu­lated out of the con­cen­tra­tion of NaCl in the sam­ples, the total vol­ume of gas­tric con­tents and the ini­tial NaCl con­cen­tra­tion in the test meal ac­cord­ing to equa­tion 9 (see addend­um). At every in­ter­val (Tₐ and Tₙ) a 1 ml sam­ple was taken. To ob­tain the phenol red con­cen­tra­tion an al­li­quet of every sam­ple was di­luted 20 fold with a NaOH/NaHCO₃ buffer (con­cen­tra­tion 0.25/0.50 mol/l) and mea­sured using a Bausch and Lomb 1001 spec­tro­phot­o­meter at 560 nm. The NaCl con­cen­tra­tion was ob­tained by mea­suring the so­dium con­cen­tra­tion us­ing an IIL 243 flame­phot­o­meter. In ad­di­tion to this the total gas­tric vol­ume and liq­uid test meal vol­ume were cal­cu­lated from the start­ing vol­ume and the chosen em­pty­ing and pro­duc­tion rates. To check the method in vivo we con­duct a num­ber of ex­per­i­ments, using Hunt’s mod­i­fi­ca­tion of George’s method as a com­par­i­son. Six­teen health­ly male vol­un­teers be­tween the age of 18 and 30 with an aver­age body weight of 75 kg par­ti­ci­pated in the ex­per­i­ment. Sub­jects ar­rived at the lab­or­a­tory in a fasted state, where a stan­dard­ised break­fast was ad­min­is­tered. A low fat com­plete nu­tri­tion­al liquid (Meri­tene® (Wander), 20 en­ergy % fat, 5 ml/kg body weight) was chosen since fat is a strong in­hibit­or of gas­tric empty­ing, and par­ticu­late mat­ter from solid food could clog the nasogastric tube. A nasogastric tube (silicon, French 14) was placed 75 min­utes after break­fast was given, the stom­ach was washed un­til no fur­ther res­i­dual was ob­served, and a re­cover­y test was done for con­trol of the pos­i­tion­ing of the tube tip. Two hours after break­fast the test meal (water, 8 ml/kg body weight, con­tain­ing 15 mg/l phenol red) was ad­min­is­tered through the tube, mixed with what­ever gas­tric con­tents, and an ini­tial sam­ple was im­medi­ately taken, so that the re­main­ing gas­tric con­tents could be ac­counted for in de­ter­min­ing ini­tial dye con­cen­tra­tion. Sam­ples were taken at in­ter­vals of 10 or 20 min­utes ac­cord­ing to sched­ule (Table 1). At every in­ter­val a sam­ple was taken, 10 ml of dye (500 mg/l) was added and mixed, and then a sec­ond sam­ple was taken. Mix­ing, sam­ple draw­ing and add­i­tion of dye was per­formed as de­scribed in the in vitro ex­per­i­ment. The total am­ount of sam­ple taken was the same as the am­ount of phenol red dye added. Using the pre­vi­ously men­tioned equa­tions both se­cre­tion and vol­ume changes could be cal­cu­lated. Dur­ing the whole test sub­jects were se­ated in a chair. Phenol red con­cen­tra­tion was mea­sured in the same way as in the in vitro ex­per­i­ment, Chloride was mea­sured conducto­met­rically us­ing an Eppendorf Chlorid­meter 6610.

Table 1 In vivo com­par­i­son of Hunt’s method and pro­posed method (aver­age of 16 sub­jects)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Vₐ₁, George’s method</th>
<th>Vₐ₁, Hunt’s method</th>
<th>Vₐ₁, Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>sd</td>
<td>ml</td>
</tr>
<tr>
<td>0</td>
<td>701</td>
<td>98</td>
<td>663</td>
</tr>
<tr>
<td>10</td>
<td>289</td>
<td>136</td>
<td>252</td>
</tr>
<tr>
<td>20</td>
<td>134</td>
<td>79</td>
<td>111</td>
</tr>
<tr>
<td>30</td>
<td>87</td>
<td>64</td>
<td>59</td>
</tr>
<tr>
<td>40</td>
<td>42</td>
<td>56</td>
<td>26</td>
</tr>
<tr>
<td>60</td>
<td>22</td>
<td>36</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2 In vitro eval­u­a­tion of George’s method and pro­posed method for cal­cu­la­tion of test meal vol­umes

<table>
<thead>
<tr>
<th>Interval</th>
<th>Vₐ₁, ml</th>
<th>Vₐ₁, Hunt’s method</th>
<th>Vₐ₁, Proposed method</th>
<th>Concentration</th>
<th>NaCl</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁b</td>
<td>600</td>
<td>600</td>
<td>15-00</td>
<td>146-0</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>T₁a</td>
<td>648</td>
<td>599</td>
<td>14-14</td>
<td>135-0</td>
<td>635</td>
<td>599</td>
</tr>
<tr>
<td>T₁b</td>
<td>567</td>
<td>507</td>
<td>13-33</td>
<td>129-5</td>
<td>536</td>
<td>476</td>
</tr>
<tr>
<td>T₁a</td>
<td>490</td>
<td>418</td>
<td>16-50</td>
<td>125-0</td>
<td>455</td>
<td>374</td>
</tr>
<tr>
<td>T₁b</td>
<td>413</td>
<td>333</td>
<td>21-00</td>
<td>118-5</td>
<td>410</td>
<td>326</td>
</tr>
<tr>
<td>T₁a</td>
<td>336</td>
<td>253</td>
<td>26-08</td>
<td>112-5</td>
<td>306</td>
<td>237</td>
</tr>
<tr>
<td>T₁a</td>
<td>336</td>
<td>253</td>
<td>26-08</td>
<td>112-5</td>
<td>306</td>
<td>237</td>
</tr>
</tbody>
</table>

phr* = phenol red.
Results

The results of the in vitro experiment are presented in Table 2. These data show that in an in vitro experiment (a) it is possible to measure the volume of a liquid test meal and total gastric contents within an accuracy of 10%, which is in accordance with George, and (b) adding a second marker and calculating test meal volume from its dilution gives no greater accuracy than calculating test meal volume from one marker only.

The results of the in vivo experiment concerning emptying rate of whole stomach contents, net test meal outflow and gastric secretion are presented in Table 1.

Discussion

Regression analysis (Figure) of the data of the in vivo experiment shows a high degree of linearity between the proposed method and Hunt’s method, but when paired t test was done a significant difference was found for every value of the curve. A possible explanation might be an overestimation of the test meal volumes by Hunt’s method. This is suggested by the very first samples taken in the test (see Table 1). The average body weight of the subjects was 75 kg, so an average test meal volume of 600 ml was given (8 ml/kg body weight) at the onset of the test. With Hunt’s method a starting volume of 663 ml is found, an overestimation of 10%.

We conclude that the results of the proposed method are comparable with those of Hunt’s method and may give an even better estimate of the volume of a liquid test meal present at any time in the stomach. Moreover, the proposed method is simple to do as a second marker is not necessary and no further assumptions and measurements are needed other than those for George’s method.

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Addendum

\begin{align*}
V_{nb}^v & : \text{volume of gastric contents before adding dye (ml)} \\
V_{na}^v & : \text{volume of gastric contents after adding dye (ml)} \\
V_{n}^i & : \text{volume of dye added (ml)} \\
V_{n}^t & : \text{volume of test meal (ml)} \\
C_{nb}^s & : \text{dye concentration in gastric contents before adding dye (mg/ml)} \\
C_{na}^s & : \text{dye concentration in gastric contents after adding dye (mg/ml)} \\
C_{n}^i & : \text{dye concentration in volume of dye added (mg/ml)} \\
A_{nb} & : \text{amount of dye in gastric contents before adding dye (mg)} \\
A_{na} & : \text{amount of dye in gastric contents after adding dye (mg)}
\end{align*}
Determination of total gastric volume, gastric secretion and residual meal using the technique of George

Calculation of gastric volume:
\[(V_{\text{snb}} + V_i)n \cdot C_{\text{sn}} = V_{\text{snb}} - C_{\text{sn}} + V_i \cdot C_i \quad (\text{eq 1})\]
\[V_{\text{snb}} = V_i n - C_{\text{sn}} - C_{\text{snb}} \quad (\text{eq 2})\]

Calculation of test meal volume:
\[A_{1a} = V_{1a} \cdot C_{1a} \quad (\text{eq 3})\]
\[A_{2b} = V_{2b} \cdot C_{2b} \quad (\text{eq 4})\]
\[V_1 = \frac{A_{2b}}{A_{1a}} \cdot V_1 \quad (\text{eq 5})\]
\[V_3 = \frac{A_{3b}}{A_{2a}} \cdot V_2 \quad (\text{eq 6})\]

Combining eq 5 with eq 6 yields
\[V_1 = \frac{A_{2b}}{A_{1a}} \cdot V_1 \quad (\text{eq 7})\]
or in general:
\[V_n = \frac{A_{nb}}{A_{(n-1)a}} \cdots \frac{A_{2b}}{A_{1a}} \cdot V_{1n} \quad (\text{eq 8})\]

Calculation of test meal volume out of NaCl dilution:
\[V_n = \frac{[\text{NaCl}]_{\text{sample}}}{[\text{NaCl}]_{\text{initial}}} \cdot V_{\text{snb}} \quad (\text{eq 9})\]

References
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