

Measuring gastric volumes by dye dilution

A HURWITZ*

From the Department of Clinical Pharmacology, University of Kansas Medical Center, Kansas City, Kansas, USA

SUMMARY The double sampling method for measuring gastric volumes is analysed mathematically. It is shown to cause magnification of randomly occurring analytical errors in dye concentration. The errors which arise in calculated gastric volume depend on extent of analytical error, concentration of dye initially placed in the stomach, repetition of analysis cycle, and ratio of volume of added dye to gastric volume. By starting with dye-free water and doubling the concentration of dye added in serial volume determinations the error in calculated volume is markedly reduced and no longer increases with successive measurements. These modifications reduce the limitations inherent in the method as originally described.

The double sampling method for measuring gastric volumes has been widely accepted and used since it was popularised by George in 1968.¹ More than 100 references in the literature have cited this report, attesting to the simplicity and convenience of the method. Nevertheless, despite scrupulous attention to precise experimental technique, certain limitations in the method as described by George make it susceptible to gross errors. In the following analysis the sources of these potential errors are identified and suggestions made for reducing the extent by which calculated gastric volume deviates from the actual volume. Improved results with a modified method are demonstrated in a model system, confirming the usefulness of the double dye dilution technique.

George¹ stated that 'the volume of fluid in a container can be ascertained by determining the increase in concentration of a dye produced by the addition of a small concentrated measure of the same dye'. Changes in gastric volume can be followed by repeating the following cycle: remove aliquot from stomach, add concentrated dye, mix gastric contents, and remove second aliquot. Gastric volumes are calculated by George's equation, thus:

$$\text{Volume}_{\text{stomach}} = \text{Volume}_{\text{added dye}} \frac{\text{Conc.}_{\text{dye}} - \text{Conc.}_{\text{after}}}{\text{Conc.}_{\text{after}} - \text{Conc.}_{\text{before}}}$$

This method is mathematically sound and convenient. It results in calculated volumes which match true volumes if all aliquots are added and removed precisely and all concentrations are determined with absolute accuracy. George's equation, as noted above, implies that errors in calculated volume are proportional to the error in volume of added dye and are very sensitive to errors in the determination of $\text{Conc.}_{\text{after}}$ because a value that is too high reduces the numerator and increases the denominator, whereas one that is too low does the reverse. The equation does not indicate that the progressive increase in gastric dye concentration which results from repeated cycles of volume determination causes enormous magnification of small inaccuracies in measured concentration and intolerable errors in calculated volume. Although this magnification is due to accumulation of dye in the stomach, the error does not depend on prior inaccuracy and is therefore not cumulative. After several accurate volume determinations a slight error in dye assay results in a large error in calculated volume which, in turn, will not prejudice the accuracy of subsequent determinations. The extent of these errors can best be defined by mathematical analysis of George's equation. The results of this analysis are summarised in the Appendix from which the following figures have been derived.

Analysis of double sampling method

The effects of changing volumes and concentrations are systematically explored. Even if all prior volume determinations were accurate, a 4% error in post

*Address for correspondence and reprint requests: Aryeh Hurwitz, MD, Clinical Pharmacology, University of Kansas Medical Center, Kansas City, Kansas 66103, USA
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Fig. 1 Predicted effect of serial gastric volume determinations on magnification of analytical error in calculating gastric volume. k (ratio of gastric volume to volume of added dye) is kept constant at 50. Initial test meal is dye-free. e is analytical error. (From Equation 3.)

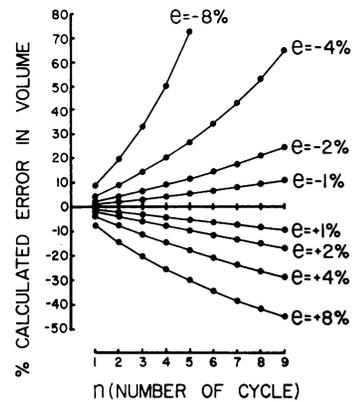


Fig. 2 Predicted effect of varying ratios of gastric volume to volume of added dye (k) on calculated error in gastric volume. Amounts of dye added and present in stomach kept constant while concentrations are varied. Number of cycle (n) is 5. e is percent analytical error. (From Equation 3).

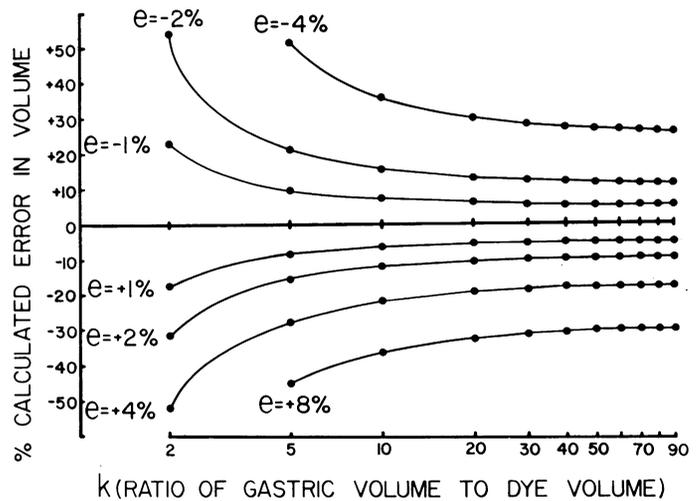
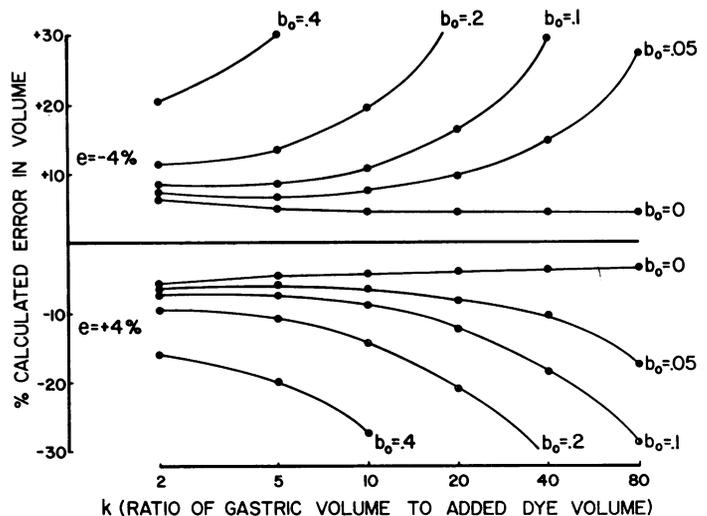


Fig. 3 Predicted effect of varying ratios of initial gastric dye concentration to concentration of dye in first added aliquot (b_0) on calculated error in first determination of gastric volume ($n=1$). Ratio of gastric volume to volume of added dye (k) varied from 2 to 80. Analytical error (e) set at +4% and -4%. (From Equation 3).



addition dye concentration in the sixth cycle would result in a 40% volume error (Fig. 1). Although large aliquots are less susceptible to technical inaccuracy, the addition of a constant amount of dye in a volume which is large relative to gastric volume causes any error in post-addition concentration to be magnified (Fig. 2). By keeping the volume of added dye to less than one-tenth the gastric volume this effect is minimised. Another means for limiting error magnification would be reduction of dye concentration in the initially ingested test meal (Fig. 3). If, in

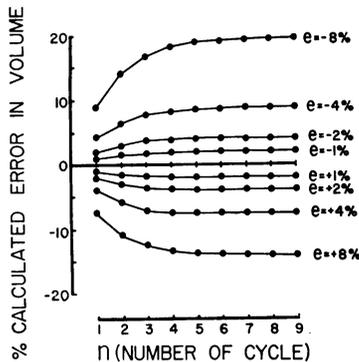


Fig. 4 Predicted effect of serial volume determinations on calculated volume error when concentration of added dye is doubled in each cycle. Ratio of gastric volume to volume of added dye (k) is 50. Initial test meal is dye-free. e is analytical error. (From Equation 6.)

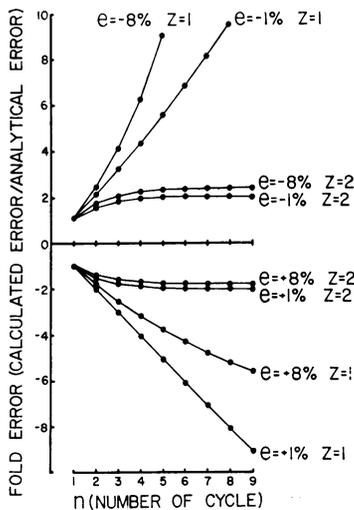


Fig. 5 Comparison of constant ($Z=1$) versus doubling ($Z=2$) concentrations of added dye on predicted serial magnification of calculated error of gastric volume. k is 50. Initial test meal is dye-free ($b_0=0$). e is analytical error. (From Equations 3 and 6.)

the first cycle, the volumes and concentrations recommended by George¹ were to be used, a 4% error in post-addition concentration would yield a volume error greater than 30%. If dye were omitted from the initial meal the volume error would be reduced by six-fold (Fig. 3).

After the first volume determination progressive error magnification occurs with each repetition of the cycle (Fig. 1). Although this can be lessened somewhat by changing the volumes of added dye (Fig. 2), this manipulation is limited by practical considerations. An easier manoeuvre is to increase the concentration of dye added in each successive cycle. This can be done conveniently by serially raising the concentration of added dye by a constant multiplier (Z). If the concentration is doubled in each successive cycle ($Z=2$), error magnification plateaus (Fig. 4). Even by the ninth cycle a 4% concentration error translates into an 8% volume error in contrast to the 65% error in the original method. A direct comparison showing the marked reduction in error magnification by serial doubling of the concentration of added dye is provided in Fig. 5.

The foregoing analysis has dealt with errors in calculated volume arising from discrepancies in the second concentration, after addition and mixing of dye. This determination is most susceptible to

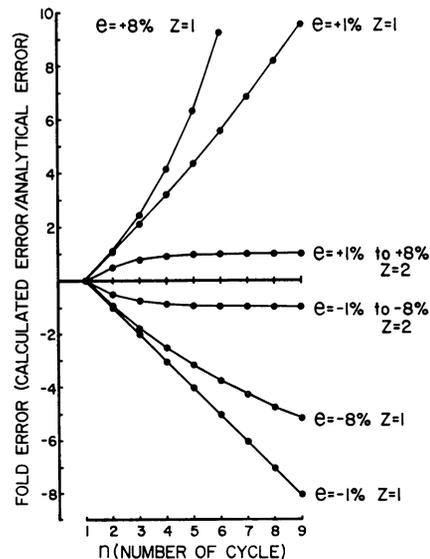


Fig. 6 Predicted effect of analytical error in gastric dye concentration before addition of concentrated dye (b_{n-1}) on serial magnification of calculated errors in gastric volume. Ratio of gastric volume to volume of added dye (k) is kept constant at 50. Initial test meal is dye-free. e is percent analytical error. (From Equations 4 and 7.)

technical errors. Errors in the first pre-addition concentration also cause progressive error magnification, which is similarly reduced by serially doubling the concentration of added dye (Fig. 6). In contrast, errors in the difference between pre- and post-addition concentration (denominator in George's equation¹) are not magnified (Fig. 7).

These considerations of concentration and volume

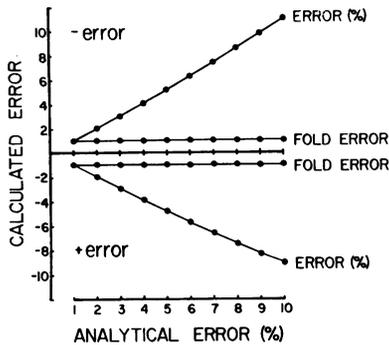


Fig. 7 Predicted effect of analytical error in difference between pre- and post-addition dye concentration ($b_n - b_{n-1}$) on calculated errors in gastric volume. (From Equation 5.)

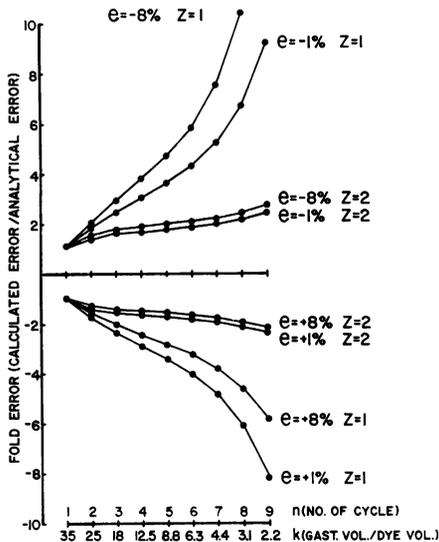


Fig. 8 Predicted effect of 'emptying' on error magnification. Simulated double dye dilution performed at regular intervals twice in each half emptying time. Gastric volume to added dye volume ratio (k) and gastric dye content reduced in parallel. Concentration of added dye kept constant ($Z=1$) or doubled in each cycle ($Z=2$). Initial k set at 50 and initial gastric dye concentration set at 0. e is percent analytical error. (From Equations 3 and 6.)

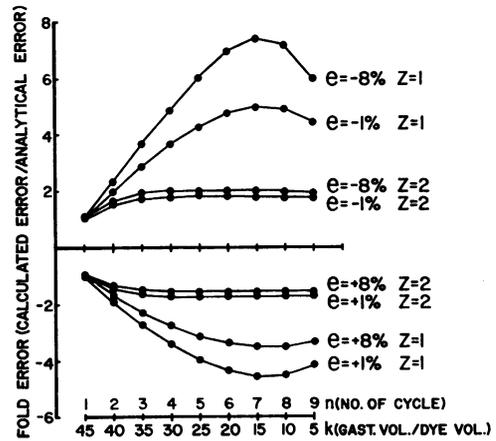


Fig. 9 Predicted effect of 'emptying' on error magnification. Double dye dilution performed at intervals to simulate emptying of one-tenth of initial volume. (From Equations 3 and 6.)

Table 1 Procedure for determining gastric volume by serial dye dilution

	Original	Modified
Initial phenol red conc. (mg/dl)	3	0
Dye aliquot added (ml)	20, 15, 10, 10, 10, 5	20
Conc. of added aliquot (mg/dl)	50	6.25, 12.5, 25, 50, 100, 200
Measuring device for aliquot	Pipette	20 ml syringe

relations in a static stomach can be generalised to include effects of secretion and emptying on error magnification. Secretion dilutes gastric dye, raising the ratio of gastric volume to added volume (k in Fig. 2). This would cause a slight reduction in error magnification. Gastric emptying would also reduce error magnification somewhat. If emptying has an exponential course² and volumes are determined twice in each half emptying time, error magnification is as in Fig. 8. Serial doubling of concentration of added dye reduces the errors as shown in this figure. If emptying is linear³ the relationships are as in Fig. 9.

Experimental methods

On the basis of this mathematical analysis and practical considerations the original method of George was modified (Table 1) and the two procedures were compared in a model system. A one litre volumetric flask and nasogastric tube (12 French, 50 in.) were used. For determining the volume of 'gastric' contents the following procedure was carried out. The flask contents was mixed by

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Table 2 Volumes and dye distribution in volume determination

	Gastric volume	Gastric dye content
Initial	V_1	V_1C_1
Sample 1 (1/2 V_a)	$V_1 - \frac{1}{2} V_a$	$(V_1 - \frac{1}{2} V_a)C_1$
Added dye (V_a)	$V_1 + \frac{1}{2} V_a$	$(V_1 - \frac{1}{2} V_a)C_1 + V_aC_a$
Sample 2 (1/2 V_a)	V_1	$= (V_1 + \frac{1}{2} V_a)C_2$
		V_1C_2

rapidly withdrawing and reinserting syringe-fuls of liquid through the tube for 45 seconds. A sample was then removed for assay and an aliquot of dye of twice its volume was added. After similar mixing a second sample equal in volume to the first was removed. Thus, there was no net change in volume in the flask (Table 2). Initially 600 ml was placed in the flask. After each volume determination 100 ml of liquid was removed and 25 ml 0.1N hydrochloric acid added to simulate emptying and secretion. The flask was then vigorously swirled to assure thorough mixing of its contents before the next volume determination. After six cycles of volume determination and reduction the contents of the flask was measured in a graduated cylinder and found to be within 5 ml of the predicted value of 225 ml. Phenol red solution was filtered before use. For the original sequence a solution of 50 mg/dl was used. In the modified method a solution of 200 mg/dl was prepared and then serially diluted with equal volumes of water to make solutions of 100, 50, 25, 12.5, and 6.25 mg/dl. The lowest concentration was used first for volume determination and progressively higher concentrations were used subsequently. Phenol red concentration was assayed after dilution with trisodium phosphate solution, 27.5 g/l, in a Gilford

spectrophotometer, Model 240, at 560 nm. Experimental data were analysed statistically by the Wilcoxon rank sum test. Data are means \pm SEM except for the exceptions noted.

Results

Although the sampling procedure as described is essentially identical with that of George, a more precise calculation of volume is represented by equation 9 (in Appendix) instead of George's equation.⁴ The mean volumes calculated by equation 9 when determined by either the original or modified procedure deviate somewhat from measured values in the flask (Fig. 10). However, the original method resulted in individual values with calculated errors which far exceeded the findings with the modified method. In four of the six series by the original method at least one calculated volume showed an increase between determinations despite a net drop of 75 ml. Each of the modified series showed a consistent drop as seen by the absence of overlapping ranges in Fig. 10. By the original method there were calculated errors (absolute) approaching 30% (Fig. 11) and more than half of the 36 values deviated from actual volume by over 10%. By the modified procedure no value deviated from measured by more than 10%. The concentrations expected in 'gastric' contents after dye addition, concentration₂, based on measured volumes were calculated by equation 8. The small random analytical errors in post-addition concentration of dye were similar by both the original and modified procedures ($2.58 \pm 0.37\%$ vs $3.43 \pm 0.28\%$, respectively). However, the mean and maximal errors in calculated volumes were much higher with the original method (11.46

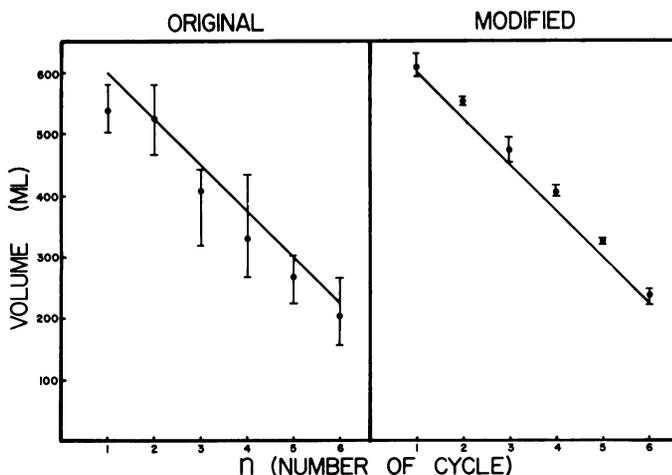
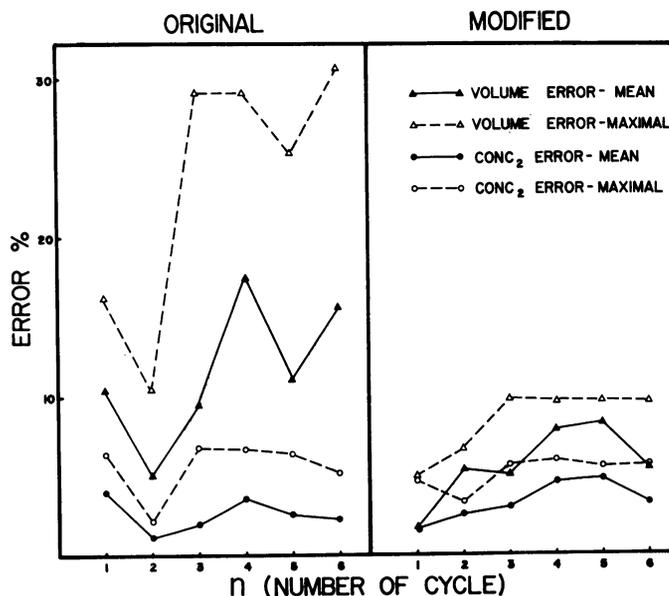


Fig. 10 Calculated volumes of liquid in flask model. Diagonal lines are identity lines for measured volumes. Data are means \pm range. Number of determinations=6 in each group. (From Equation 9.)

Fig. 11 Calculated errors in volumes and concentrations in flask model. Data calculated by Equations 8 and 9. Number of determinations=6 in each group.



$\pm 1.49\%$ vs $5.75 \pm 0.52\%$, $n=36$ in each group, $P < 0.01$) as shown in Fig. 11. The maximal volume errors were reduced by about three-fold by the modified procedure. The theoretical predictions of error magnification in Figs. 3 and 9 are confirmed as shown in Fig. 12.

Discussion

Techniques employing dilution of markers have been used for many years to study gastric volumes, emptying and secretion.⁵ Critical to all these studies is the accurate determination of gastric volumes. Hollander and Glickstein⁶ in studying gastric secretion noted that 'a compounding of analytical errors arises entirely from the arithmetical manipulation of the data and is inherent in the dilution indicator technique itself'. Such analysis has not previously been done on the dye dilution method for measuring gastric volumes. The foregoing figures and experimental model demonstrate the magnification of errors inherent in the design of the double sampling method for measuring gastric volumes. The errors may become enormous. They will occur because of technical imprecision in dye addition, mixing, or assay. Each calculated error arises independently of the accuracy of prior or subsequent determinations and depends on the random occurrence of a technical error. Although the expansion of errors is due to the cyclic nature of the technique, the errors are not cumulative and may occur in any cycle.

These errors have been noted previously. George, who popularised the method, reported errors of up to 9% in a static glass model *in vitro*. As he noted such errors under ideal conditions it is not surprising that similar findings would arise in experimental situations. It is very difficult to identify such errors or determine their extent and frequency in intact

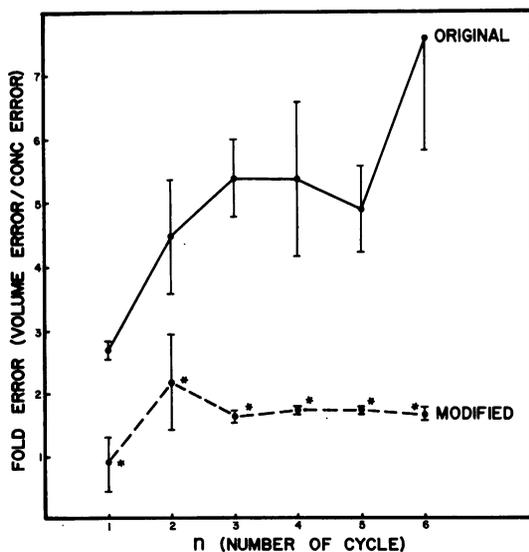


Fig. 12 Magnification of concentration error in volume calculation in flask model. Data calculated by Equations 8 and 9 and expressed as means \pm S.D. Number of determinations=six in each group, * $P < 0.01$.

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animals or people. Nevertheless, one group which used the method admit that they found inconsistencies.⁷ They state that 'when a single point was grossly out of line with the other values and excessively biasing the results, this point was rejected and analysis was based on the remaining points'. Hildes and Dunlop,⁸ who used double dye dilution for measuring gastric volumes nearly two decades before George, occasionally found an increase in phenol red concentration during a 15 to 20 minute period. They attributed these findings to inadequate mixing of the dye with stomach contents. Another study⁹ also found that 'the calculated amount of phenol red in the stomach occasionally increases during a 20-minute period'. These authors ascribed their finding to duodenal regurgitation during emptying rather than to technical or analytical difficulties. Irrespective of the cause, such changes would reduce the numerical value of the denominator in George's equation, predisposing to errors in the calculated volumes.

In his original description George suggested that larger volumes of dye be added in the early determinations. This approach of adding more dye at the start when it is needed least would predispose to greater errors. One year later Cavell¹⁰ recognised the error problem and suggested raising the concentration of non-absorbable marker in later cycles of volume determination. His report did not indicate the extent of error nor substantiate the small arbitrary increments in concentration which he used. The recommendations of Cavell have been largely ignored in subsequent reports. Unless errors have been of such magnitude as to be absolutely impossible, data based on double sampling have been uncritically accepted, as no independent verification of gastric volumes is readily available.

The importance of reducing the dye concentration of the instilled meal is illustrated in Fig. 3 and was demonstrated experimentally (Fig. 12). However, the study of Bloom *et al.*¹¹ demonstrated that a significant amount of phenol red is bound in the non-secreting canine gastric pouch during the first 15 minutes after instillation. Loss during a rapid (one minute) mixing procedure in a secreting organ is unknown. Nevertheless, as these authors recommend, actual dye recovery should be studied and gastric washing with dye solution may be advisable. Then dye-free solution can be placed in the stomach at the start of an emptying study.

The modified procedure which is proposed to reduce error magnification does not interfere with calculation of gastric secretion by dye dilution^{4, 8} or chloride titration.¹² If needed, the calculations of Dubois *et al.*⁴ may be adapted to this method to correct for changes due to secretion and emptying

during the brief sampling and dilution interval. When the modified procedure was compared with the original method, the mathematically predicted advantages were confirmed. Despite meticulous attention to detail and ideal conditions in the model system, random analytical errors approaching 7% were found in both methods. Syringes, which are more convenient than the pipettes recommended by George, resulted in no greater analytical error. However, these relatively minor errors were translated to volume errors exceeding 130 ml by the original method, whereas the largest error in the modified method was 44 ml. In the absence of emptying and dilution (secretion) this discrepancy in extent of volume error would have been even greater. By omitting dye from the water initially placed in the stomach and doubling the dye concentration in each serial addition there is marked reduction of volume error. Also, a set of solutions is conveniently provided for a standard curve for the assay. As this procedure eliminates the progressively enlarging error inherent in the original method, the double dye dilution procedure becomes a feasible method for determining gastric emptying. If additional data points are needed, increasing concentrations of a second non-absorbed marker may be used. Polyethylene glycol, which does not interfere with dye determination, may be used.

The advice and suggestions of Dr Allan R Cooke were most helpful. The invaluable assistance of Russell Moore, who calculated the numerical values on a Texas Instrument SR52 calculator, is greatly appreciated.

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Appendix †

SYMBOLS

The symbols used in this Appendix are as follows:

- n = number of volume determination cycle
- V_n = volume at nth cycle
- C_n = concentration of gastric dye at end of nth cycle
- V_a = volume of added dye
- C_a = concentration of dye added in first cycle
- b_n = C_n/C_a
- k = V_n/V_a
- Z = multiplier of concentration of added dye
- e = fractional error

EQUATIONS

If the concentration of dye added in the first cycle, C_a, is multiplied by a constant multiplier, Z, in each subsequent cycle, then, in the nth cycle

$$V_n C_n = V_{n-1} C_{n-1} + Z^{n-1} C_a V_a$$

$$\frac{V_{n-1}}{V_a} = \frac{Z^{n-1} C_a - C_n}{C_n - C_{n-1}} = \frac{Z^{n-1} - C_n/C_a}{C_n/C_a - C_{n-1}/C_a}$$

With no secretion or emptying, letting

$$k = \frac{V_n}{V_a} = \frac{V_{n-1}}{V_a}; b_n = \frac{C_n}{C_a}; b_{n-1} = \frac{C_{n-1}}{C_a}$$

then

$$\text{Eq. 1} \quad k = \frac{Z^{n-1} - b_n}{b_n - b_{n-1}}$$

Solving for b_n

$$b_n = \frac{Z^{n-1}}{k+1} + \left(\frac{k}{k+1}\right) b_{n-1}$$

†More detailed derivations of equations are available from the author on request.

Letting b₀ represent the ratio of dye concentration in test meal to that in the first added aliquot, then after repeating the cycle of volume determination n times.

$$b_n = \sum_{j=1}^n \frac{Z^{n-j} k^{j-1}}{(k+1)^j} + \left(\frac{k}{k+1}\right)^n b_0$$

which can be expressed as

$$\text{Eq. 2} \quad b_n = \frac{Z^n - [k/(k+1)]^n}{Z(k+1) - k} + \left(\frac{k}{k+1}\right)^n \cdot b_0$$

If Z=1 (constant concentration of added dye as in the original method of George), then

$$b_n = 1 - (1 - b_0) \left(\frac{k}{k+1}\right)^n$$

Introducing an error, e, so b_n' = (1 + e)b_n

$$\text{Eq. 3} \quad k' = \frac{1 - b_n'}{b_n' - b_{n-1}'} = \frac{(1 - b_0)k^n - e[(k+1)^n - (1 - b_0)k^n]}{(1 - b_0)k^{n-1} + e[(k+1)^n - (1 - b_0)k^n]}$$

If the error were to be in b_{n-1}, then

$$\text{Eq. 4} \quad k' = \frac{1 - b_n}{b_n - b_{n-1}'} = \frac{k^n (1 - b_0)}{k^{n-1}(1 - b_0) + e(k+1)[k^{n-1}(1 - b_0) - (k+1)^{n-1}]}$$

If the entire denominator term is in error,

$$\text{Eq. 5} \quad k' = \frac{Z^{n-1} - b_n}{(1 + e)(b_n - b_{n-1})} = \left(\frac{1}{1 + e}\right) k$$

If dye is omitted from the meal initially placed in the stomach (b₀=0) and concentration of added dye is doubled in each cycle (Z=2) as in the modified method, then from

$$\text{Eq. 2} \quad b_n = \frac{2^n - [k/(k+1)]^n}{k+2}$$

If an error occurs in b_n so b_n' = (1 + e)b_n

$$\text{Eq. 6} \quad k' = \frac{2^{n-1} - b_n'}{b_n' - b_{n-1}'} = \frac{k[2^{n-1}(k+1)^n + k^{n-1}] - e[2^n(k+1)^n - k^n]}{[2^{n-1}(k+1)^n + k^{n-1}] + e[2^n(k+1)^n - k^n]}$$

An error in b_{n-1} would result in

$$\text{Eq. 7} \quad k' = \frac{2^{n-1} - b_n}{b_n - b_{n-1}'} = \frac{k[2^{n-1}(k+1)^n + k^{n-1}]}{[2^{n-1}(k+1)^n + k^{n-1}] + e(k+1)[k^{n-1} - (2k+2)^{n-1}]}$$

The error in k is

$$\left(\frac{k'}{k} - 1\right) \times 100 = \% \text{ calculated error in volume.}$$

ANALYSIS OF EXPERIMENTAL DATA

The foregoing analyses have not corrected for the volumes of added dye or of the aliquots removed for assay. After dye addition and mixing, the amount of dye in the stomach, as indicated in Table 2, is

$$(V_{n-1} - \frac{1}{2} V_a) C_{n-1} + V_a Z^{n-1} C_a = (V_{n-1} + \frac{1}{2} V_a) C_n$$

Therefore

$$\text{Eq. 8} \quad C_n = \frac{(V_{n-1} - \frac{1}{2} V_a) C_{n-1} + V_a Z^{n-1} C_a}{V_{n-1} + \frac{1}{2} V_a}$$

and

$$\text{Eq. 9} \quad V_{n-1} = V_a \frac{Z^{n-1} C_a - C_n}{C_n - C_{n-1}} + \frac{1}{2}$$

For error analysis, with no emptying or secretion, this equation can be rearranged and prior notation introduced, thus

$$\frac{V_{n-1}}{V_a} - \frac{1}{2} = k - \frac{1}{2} = \frac{Z^{n-1} - C_n/C_a}{C_n/C_a - C_{n-1}/C_a} = \frac{Z^{n-1} - b_n}{b_n - b_{n-1}}$$

This equation is obviously similar to Eq. 1 with $(k - \frac{1}{2})$ replacing k. Therefore, an entire set of equations leading to equations like Eq. 3 through Eq. 7 can be derived. As for $k > 10$ the numerical value of k is nearly identical to $(k - \frac{1}{2})$, a series of figures nearly superimposable on Figs. 1-9 could be drawn. All the conclusions regarding error magnification are therefore identical and $Z=2$ and $b_0=0$ remain convenient means for reducing the errors in calculated volumes, as was demonstrated in the flask model.