A mixed isotope method for the study of gastric absorption of drugs

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The most widely used method for studying gastric absorption of drugs is that devised by Shanker, Shore & others (1957), which involves the measurement of the decline in concentration of drug in the pylorus-ligated stomach. In the absence of adsorption to the stomach wall, this provides a means of studying the potential for gastric absorption of a drug in relation to its lipophilicity and pKa and for comparing this potential with those of other drugs. However, the method is subject to certain limitations. Firstly, it does not permit evaluation of the significance of gastric absorption in the presence of normal gastric emptying. Secondly, the presence of an anaesthetic as an accessory to the pylorus ligation may itself influence the course of gastric absorption by altering gastric motility and blood flow to the stomach. Hence if the significance of gastric absorption of a particular drug under normal physiological conditions is to be properly evaluated, the measurement of the rate of this process should be performed in animals with a functioning pylorus and an allowance made for disappearance of drug from the stomach by gastric emptying.

Several workers have described the use of non-absorbable markers in absorption studies to make an allowance for loss of drug by gastric emptying and also for dilution of stomach contents by gastric secretion. For example, Cooke & Hunt (1970) used the marker phenol red when studying the gastric absorption of acetylsalicylic acid in man. The method relies on the principle that changes in drug concentration due to gastric emptying and dilution of stomach contents parallel similar changes in the concentration of the marker. Any additional decline in drug concentration in gastric contents must then result from gastric absorption. In previously reported studies, the non-absorbable marker was unlabelled and was determined spectrophotometrically by an assay separate from that used to measure concentrations of drug in stomach contents. The low sensitivities of the assay methods necessitated the use of relatively high concentrations of non-absorbable markers. Under these conditions, the method is subject to the criticism that the marker itself may influence the absorption of the drug (Beerman, Groschinsky-Grind & Rosen, 1976).

The present communication describes a modification which simplifies the procedure when radiolabelled drug is available. The ratio of concentration of a ^{14}C-labelled drug to that of a ^{3}H-labelled marker is used to make automatic compensation for effects of volume changes and gastric emptying without the need for separate assays. The ratio of $^{14}C/^{3}H$ in gastric contents remains constant, despite gastric secretion and gastric emptying, unless drug is absorbed from the stomach. In this case, the $^{14}C/^{3}H$ ratio declines at a rate equal to the rate of gastric absorption of the drug. Furthermore, simultaneous determination of the gastric emptying rate allows calculation of the total percentage of the dose absorbed from the stomach.

The drug chosen for study of gastric absorption by this method was [^{14}C]salicylic acid, since Shanker & others (1957) had shown it to be well-absorbed from the pylorus-ligated stomach of the rat, and not significantly adsorbed to stomach wall. The marker was [^{3}H]inulin, an inert substance negligibly absorbed from the gastrointestinal tract (Miller & Schedl, 1970), and not subject to significant exchange of tritium with water under physiological conditions (Marlow & Sheppard, 1970). The animals used were one male and two female patas monkeys (Shamrock Farms Ltd., Brighton, Sussex), 3·0–3·5 kg, fasted overnight. The dose (0·5 mg kg^{-1}) of salicylic acid was administered by oral intubation such that each animal received 5 ml kg^{-1} of a solution containing 100 μg ml^{-1} [^{14}C]-salicylic acid and 1 μg ml^{-1} [^{3}H]inulin (The Radiochemical Centre, Amersham, Bucks) in isotonic saline. Immediately after dosing and subsequently at 10 min intervals for 1 h, aliquots of approximately 1 ml of gastric contents were aspirated and after the hour the entire stomach contents were aspirated. At the end of the period of serial sampling, stomach contents were aspirated so that the amount of remaining [^{3}H]inulin could be assayed. Because of the possibility of incomplete recovery of stomach contents in the final gastric wash, the efficiency of this recovery was checked by introduction of [^{14}C]polyethylene glycol (PEG 4000) (^{14}C-PEG) (The Radiochemical Centre).

^{14}C-PEG (8·15 μ Ci) was administered by oral intubation of 20 ml of a 12 μg ml^{-1} solution in isotonic saline, and was recovered within 3 min of dosing by washing out with 3 × 50 ml isotonic saline. Radioactivity administered as ^{14}C-PEG 4000 was in large excess over that of any remaining [^{14}C]salicylic acid, which thus did not interfere with the determination of the ^{14}C-labelled marker. Mixing of ^{14}C-PEG 4000 and [^{3}H]inulin in stomach contents was assumed to have been achieved by the regular contractions of the stomach and by subsequent washes. Three 50 ml portions of isotonic saline were introduced into the stomach and the gastric contents aspirated after each wash. Recoveries of ^{14}C-PEG 4000, determined in 2 animals only, were 90 and 101%. Consequently no compensation for loss of gastric contents was considered necessary. Samples of gastric contents and the pooled gastric washes were centrifuged to precipitate any solid particles. Tritium and ^{14}C concentrations in suitable aliquots were assayed

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by liquid scintillation counting under conditions in which the two isotopes could be separately determined.

The half-life of gastric absorption was calculated from the slope of the regression line relating $\log_{10}$ $^{14}C/^{3}H$ ratio to times after dosing. The gastric emptying half-life was determined from the amount of [^{3}H]-inulin not emptied from the stomach in the 60 min after dosing. The calculation assumed monoexponential gastric emptying, as established by Hunt & MacDonald (1954) from studies in man. The method of calculation used the formula:

$$\text{Gastric emptying } t^{1/2} = \frac{0{\cdot}301\ t}{\log_{10}\frac{(Xo)}{(Xt)}}$$

The total percentage of the administered dose absorbed from the stomach was calculated from the rate constants for gastric absorption and gastric emptying, k_{as} and k_{ge} respectively, as follows:

$$\text{Percentage dose} = \frac{k_{as}}{k_{as} + k_{ge}} \times 100\%, \text{ where } k = \frac{0{\cdot}693}{t^{1/2}}$$

As illustrated in Fig. 1, the ratio of [^{14}C]salicylic acid to [^{3}H]inulin declined monoexponentially with time (correlation coefficient = −0·996), indicating that substantial gastric absorption of salicylic acid had taken place. The mean gastric absorption half-life (± s.e.m.) was 12·8 ± 1·8 min. The mean gastric emptying half-life (± s.e.m.) was 37·3 ± 9·0 min, in good agreement with the value of 30 min previously reported by Franklin (1975). The mean percentage of the administered dose absorbed from the stomach (± s.e.m.) was 69·0 ± 6·1%.

Thus, this mixed isotope ratio method provides a simple means of determining the rate of gastric absorption of a drug without recourse to artificial preparations involving surgical ligation of the pylorus. Whilst there are existing methods to compensate for changes in drug concentration resulting solely from gastric secretion and gastric emptying, this method confers the greater convenience of a single assay by comparison with the more usual determination of drug and non-absorbable marker by separate specific assays. Finally, the method incorporates the determination of gastric emptying simultaneous with that of absorption, thus permitting a simple assessment of the total extent to which gastric absorption occurs under conditions of unimpaired gastric emptying.

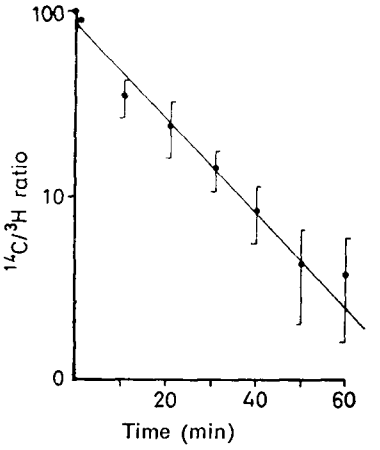

FIG. 1. The gastric absorption of [^{14}C]salicylic acid (0·5 mg kg^{-1}) in patas monkeys. One male and two female animals each received by oral intubation 5 ml kg^{-1} of a solution of 100 μg ml^{-1} [^{14}C]salicylic and 1 μg ml^{-1} [^{3}H]inulin. The ratio of ^{14}C to ^{3}H in gastric contents was measured at intervals from 0–60 min after dosing and values were expressed as a percentage of the ratio in the dose before administration. Each result is the mean ± s.e.m. of determination in the 3 animals.

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