THE USE OF A DEUTERIUM TRACER TECHNIQUE TO FOLLOW THE FATE OF FLUIDS INGESTED BY HUMAN SUBJECTS: EFFECTS OF DRINK VOLUME AND TRACER CONCENTRATION AND CONTENT

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SUMMARY

Deuterium oxide (\(^2\)H\(_2\)O) has been added to drinks as a tracer for water to estimate the availability to the body water pool of ingested fluids, but doubts have been raised as to the reliability of the method. The present investigation evaluated the effects of systematic variations in the volume of fluid consumed and the amount and concentration of added tracer on the rate of accumulation of tracer in arterialized blood after ingestion of a labelled drink. Three separate experiments were undertaken. In expt 1, six healthy men ingested on separate occasions 200, 400 and 800 ml of a dilute glucose−electrolyte solution: all test drinks contained the same concentration (40 g l\(^{-1}\)) of \(^2\)H\(_2\)O. In expt 2, six healthy men ingested 200, 400 and 800 ml of the same glucose−electrolyte drink: each drink contained 8 g of \(^2\)H\(_2\)O so that the concentration, but not the amount, of \(^2\)H\(_2\)O differed between treatments. In expt 3, six healthy men ingested 400 ml of the same drink on three separate occasions: each drink contained 8, 16 or 32 g of tracer so that amount and concentration of \(^2\)H\(_2\)O both varied. Arterialized venous blood samples were collected for the determination of deuterium (\(^2\)H) concentration before ingestion of the test drink and at intervals for 120 min after ingestion. All trials for each of the experiments were conducted in the morning after an overnight fast and trials were in randomized order and separated by 7 days. In expt 1, the blood \(^2\)H concentration at all time points from 2 min after ingestion of the test drink onwards was higher for the drink containing 32 g \(^2\)H\(_2\)O than for the drink containing 16 g \(^2\)H\(_2\)O, which in turn was higher than after ingestion of the drink containing 8 g of \(^2\)H\(_2\)O. In expt 2, no significant differences between treatments were observed at any time. In expt 3, the rate of \(^2\)H accumulation was greater after ingestion of the drink containing 32 g of \(^2\)H\(_2\)O than after either of the other two drinks, and the \(^2\)H accumulation rate was greater after ingestion of the drink containing 16 g of \(^2\)H\(_2\)O than after the drink containing 8 g of \(^2\)H\(_2\)O. When data from all three experiments were combined, significant correlations were observed between the rate of accumulation of \(^2\)H in the circulation (p.p.m. min\(^{-1}\)) and the amount \((r_s = 0.75, P < 0.001)\) and concentration \((r_s = 0.69, P < 0.001)\) of \(^2\)H\(_2\)O in the test drink, but there was no relationship \((r_s = 0.09, P = 0.5)\) between the rate of \(^2\)H accumulation in the blood and the volume of the drink consumed. The results suggest that the rate of tracer accumulation in the blood after ingestion of different volumes of test drinks is not a reliable indication of the availability of the ingested fluid, but that the method gives at least a qualitative measure of the sum of the effects of gastric emptying and intestinal water absorption.

INTRODUCTION

Techniques that measure either gastric emptying or intestinal absorption have been used extensively to assess the fate of ingested fluids and to assess the efficacy of oral rehydration solutions intended for the treatment of diarrhoeal disease (Leiper & Maughan, 1988; Elliott, 1989; Elliott & Maughan, 1990). The present investigation was designed to evaluate the effects of adjusting the volume of fluids or the concentration of deuterium oxide as a tracer to compare the rate of accumulation of \(^2\)H\(_2\)O in the circulation of human subjects.
1989) and of sports drinks used for the improvement of athletic performance (Lamb & Brodowicz, 1986; Murray, 1987). Each of these methods, however, can provide only part of the answer, and it is not clear whether the availability of ingested fluids is limited by the rate of gastric emptying or by the rate of intestinal water uptake. A further disadvantage is that although non-invasive methods are available, quantitative measurements require the use of intubation methods. It also seems to be the case that the results obtained when using a multilumen perfusion set to make measurements of water absorption in the small intestine depend critically on the precise location of the perfusion set and on the length of the test segment used (Shi et al. 1994).

It has been proposed that the addition of a tracer for water to orally ingested fluids and the subsequent measurement of the accumulation rate of that tracer in body fluids will give a measure of the integrated effects of gastric emptying and intestinal absorption and will provide a useful measure of the availability of ingested solutions. Although a small amount of tracer exchange may occur between the stomach and the systemic circulation, the amount of net water uptake that occurs in the stomach is negligible (Scholer & Code, 1954), so both gastric emptying and intestinal absorption must occur before the tracer appears in the circulation. This method, using deuterated water ($\text{D}_2\text{H}_2\text{O}$) as a tracer, has recently been used in a number of studies in which drinks with different compositions have been compared (Davis et al. 1987) or in which the effects of exercise (Maughan et al. 1990) or differences in drink temperature (Lambert & Maughan, 1992) on the fate of ingested fluid have been measured.

Objections have been raised to the use of the tracer method as employed in the studies referred to above, as water movement in the intestine is a bi-directional process, and tracer will accumulate in the blood even when net secretion of water is occurring in the small intestine (Gisolfi et al. 1990). Nonetheless, the results of Davis et al. (1987) who compared solutions of different composition were largely those that would have been expected from the known gastric emptying and intestinal absorption characteristics of the solutions being compared. Gisolfi et al. (1992) also reported that results obtained using the tracer method were qualitatively similar to those obtained using a perfusion method to measure net water flux in the proximal small intestine.

The purpose of the present investigation was to further examine the use of the $\Delta\text{H}_2\text{O}$ tracer method to study the availability of water from ingested fluids. Increasing the volume of fluid in the stomach will increase the rate of gastric emptying, thus providing a higher rate of delivery of both tracer and water to the small intestine (Costill & Saltin, 1974; Mitchell & Voss, 1989; Noakes et al. 1991). Increasing both the volume of labelled water in the small intestine and the tracer concentration should increase the rate of appearance of tracer in the circulation.

**METHODS**

Prior approval for this study was obtained from the local ethics committee and the experiments were performed according to the principles of the Declaration of Helsinki. Before participation, each subject was informed of the potential risks and stresses associated with participation, and written consent was obtained. All subjects were healthy young men free from any history of gastrointestinal disease. Three separate experiments were undertaken, and all trials for each experiment took place after an overnight fast: trials were conducted 7 days apart and were administered in randomized order. The solution ingested for each treatment was a commercially available dilute glucose-electrolyte solution produced for use in the treatment of diarrhoeal disease. The composition of the test drink (mmol l$^{-1}$) was: glucose, 200; sodium, 35; potassium, 20; chloride, 37; bicarbonate, 18; and the total osmolality of the solution prior to the addition of the $\Delta\text{H}_2\text{O}$ tracer was 310 mosmol kg$^{-1}$. 
On the morning of the experiment, the subject was seated comfortably and immersed one hand in water maintained at a temperature of 42 °C for 10 min to allow arterialized venous blood samples to be obtained (Forster et al. 1972). A 21 g venous cannula was then placed in a superficial vein on the dorsal surface of the heated hand and a 5 ml blood sample was obtained. The subject then ingested the experimental drink as rapidly as possible. Further 5 ml arterialized venous blood samples were obtained at 2, 5, 10, 20, 30, 45, 60, 90 and 120 min after the drink was consumed. Subjects remained seated with their hand immersed in water maintained at a temperature of 42 °C for the duration of the trial.

Water (a mixture of H2O, 2H2O and 3H2O) was separated from the blood samples by vacuum distillation and 3H was measured by infra-red spectrophotometry as previously described (Lukaski & Johnson, 1985). All samples were analysed in duplicate. The deuterium accumulation rate was calculated by linear regression of the blood concentration following ingestion of the test solution to the time of maximum concentration in the circulation. Blood 2H accumulation data for each of the experiments were compared with a two-factor ANOVA with repeated measures on both treatment and time factors. Rates of blood 2H accumulation over time within each of the studies were compared using a one-way ANOVA for repeated measures. Separate Spearman’s rank correlation coefficients (r) were calculated to relate the slope values for blood 2H accumulation to the volume of drink consumed, to the 2H2O content and to the 2H2O concentration in the drinks. After analysis with the appropriate ANOVA, the Newman–Keul’s post hoc analysis was used to locate differences between pairs of means. Differences were considered significant when they achieved a probability level of 0.05 or less.

**Experiment 1**
Six men (age (mean ± s.d.) 28 ± 6 years, height 175 ± 5 cm, mass 66.2 ± 3.9 kg) participated in this experiment. On three separate occasions, in randomized order and separated by 7 days, subjects ingested a fixed volume of the glucose–electrolyte solution: the test drink volumes were 200 ml (L1), 400 ml (M1) and 800 ml (H1). The concentration of 2H2O in all three drinks was the same (4 g (100 ml)⁻¹) so the total amount ingested was 8 g for treatment L1, 16 g for treatment M1 and 32 g for treatment H1.

**Experiment 2**
Six men (age 23 ± 2 years, height 177 ± 6 cm, mass 73.3 ± 9.4 kg) were subjects for this experiment. Experimental conditions were the same as for ext 1, but the 2H2O content of each of the test drinks was the same (8 g). The volume consumed was 200 ml (L2), 400 ml (M2) or 800 ml (H2), so that the 2H2O concentration in the drinks was different: 4 g (100 ml)⁻¹ in L2, 2 g (100 ml)⁻¹ in M2 and 1 g (100 ml)⁻¹ in H2.

**Experiment 3**
Subjects for this experiment were six men (age 31 ± 7 years, height 171 ± 4 cm, mass 67.8 ± 6.1 kg). Experimental conditions were the same as for the two preceding experiments except that the volume of fluid consumed was constant (400 ml) in all trials. Solution L3 contained 8 g of 2H2O to give a concentration of 2 g (100 ml)⁻¹; solution M3 contained 16 g of 2H2O to give a concentration of 4 g (100 ml)⁻¹, and solution H3 contained 32 g of 2H2O to give a concentration on 8 g (100 ml)⁻¹.

**RESULTS**

**Experiment 1**
The blood 3H concentration increased rapidly on all treatments, and significant time (P < 0.001), treatment (P < 0.001) and interaction (P < 0.001) effects were observed. Significant differences between the three treatments in blood 2H concentrations were observed at all time points after the first 2 min sample point. The blood 2H concentration was higher on trial H1, where 800 ml of drink containing 32 g of 2H2O was ingested, than on the other two trials (Fig. 1) and was higher on M1 (16 g 2H2O in 400 ml) than on L1 (8 g in 200 ml). The calculated linear rate of blood 2H accumulation to peak concentration was significantly (P = 0.038) greater on trials H1 (33 ± 3 p.p.m. min⁻¹) and M1 (33 ± 4 p.p.m. min⁻¹) than on trial L1 (16 ± 5 p.p.m. min⁻¹).
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Fig. 1. Blood deuterium concentration (p.p.m.) after ingestion of a glucose–electrolyte drink containing \( ^2\text{H}_2\text{O} \) at a concentration of 4 g (100 ml)\(^{-1} \): drinks were consumed in a volume of 200 ml (L1), 400 ml (M1) and 800 ml (H1). Values are means \( +/− \) S.D. (\( n = 6 \)).

Fig. 2. Blood deuterium concentration (p.p.m.) after ingestion of a glucose–electrolyte drink containing 8 g of \( ^2\text{H}_2\text{O} \): drinks were consumed in a volume of 200 ml (L2), 400 ml (M2) and 800 ml (H2). Values are means (\( n = 6 \)), and the variance is shown as the S.D. of the mean for the highest and lowest values for each time point.

Experiment 2

No significant differences between the three trial conditions in blood \( ^2\text{H} \) concentration were found to exist at any time point (Fig. 2). The linear rates of blood \( ^2\text{H} \) accumulation were the same for trial L2 (11 ± 2 p.p.m. min\(^{-1} \)), M2 (11 ± 4 p.p.m. min\(^{-1} \)) and H2 (12 ± 8 p.p.m. min\(^{-1} \)).

Experiment 3

Significant time (\( P < 0.001 \)), treatment (\( P < 0.001 \)) and interaction (\( P < 0.001 \)) effects were found to exist when the three experimental conditions were compared (Fig. 3). The blood \( ^2\text{H} \) concentration on trial H3 (32 g of \( ^2\text{H}_2\text{O} \) in 400 ml) was higher than on trial M3 (16 g of
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Fig. 3. Blood deuterium concentration (p.p.m.) after ingestion of 400 ml of a glucose–electrolyte drink containing \(^2\text{H}_2\text{O}\) at a concentration of 2 g (100 ml\(^{-1}\)) (L3), 4 g (100 ml\(^{-1}\)) (M3) or 8 g (100 ml\(^{-1}\)) (H3). Values are means ± s.d. (n = 6).

Fig. 4. Combined data from expts 1, 2 and 3 showing the relationship between the blood \(^2\text{H}\) accumulation rate and the amount of \(^2\text{H}_2\text{O}\) present in the test drink. Boxplots show median, first and third quartiles, and maximum and minimum values.

\(^2\text{H}_2\text{O}\) in 400 ml) at all time points after the first 5 min of sampling, and values on trial M3 were higher than on L3 (8 g of \(^2\text{H}_2\text{O}\) in 400 ml) at all times after 2 min. Slope values were greater on H3 (59 ± 5 p.p.m. min\(^{-1}\)) than on M3 (23 ± 2 p.p.m. min\(^{-1}\)) or L3 (19 ± 3 p.p.m. min\(^{-1}\)).

When the results of all the data are compared, significant correlations were observed to exist between the blood \(^2\text{H}\) accumulation rate and the amount \((r_s = 0.75, P < 0.001; \text{Fig. 4})\) and the concentration \((r_s = 0.69, P < 0.001; \text{Fig. 5})\) of \(^2\text{H}_2\text{O}\) in the test drink. No relationship was seen between the rate of blood \(^2\text{H}\) accumulation and the volume of drink consumed \((r_s = 0.09; P = 0.50; \text{Fig. 6})\).
Fig. 5. Combined data from expts 1, 2 and 3 showing the relationship between the blood $^2$H accumulation rate and the concentration of $^2$H$_2$O present in the test drink. Boxplots show median, first and third quartiles, and maximum and minimum values.

Fig. 6. Combined data from expts 1, 2 and 3 showing the relationship between the blood $^2$H accumulation rate and the volume of test drink consumed. Boxplots show median, first and third quartiles, and maximum and minimum values.

**DISCUSSION**

The results of the three studies reported here suggest that the rate of $^2$H accumulation in arterialized blood after ingestion of a drink labelled with $^2$H$_2$O is related to the amount of tracer ingested and to its concentration in the drink, but that where these factors are constant it is not affected by the volume of fluid ingested. This indicates that, where the deuterium tracer method is to be used to assess the fate of ingested fluids, the amount and concentration of tracer used must be constant if valid results are to be obtained. Because it is not possible to keep both of these factors constant when the volume varies, the deuterium tracer method cannot be used to compare responses to the ingestion of different drink volumes.

Comparing the results of expts 1 and 2 it is apparent that, when the tracer concentration is maintained at a constant level but the volume of fluid ingested is varied (expt 1), the rate of
appearance of the tracer in the circulation follows the pattern expected: as the gastric emptying rate increases with increasing volume ingested, so the rate of accumulation in the circulation increases. The tracer concentration in all test drinks was the same, so an increased rate of appearance suggests that the rate of water absorption in the small intestine is determined largely by the rate of gastric emptying. However, when the volume of fluids ingested is varied in the same way but the total tracer content is the same (resulting in a decreasing tracer concentration as the volume of fluid ingested increases; expt 2), there is no relationship between the volume of test drink ingested and the rate of tracer accumulation in the circulation, despite the faster rate of emptying that would be expected as the volume ingested was increased. The decreased rate of intestinal tracer absorption because of the lower tracer concentration has counterbalanced the effects of an increased volume and thus an increased surface area for absorption.

In expt 3, in which the volume of fluid ingested was the same on all trials and the same subjects were used on each occasion, it is reasonable to expect that the rate of gastric emptying should have been the same on each of the three trials. The composition of the test drink was also the same on each occasion, so the rate of water uptake should be the same. There have been rather few published studies in which the day-to-day variability in these processes has been reported, but one study suggested that the daily variability for gastric emptying was about 29% (Beckers et al. 1991) and another study reported 31% daily variability for the steady state segmental jejunal perfusion technique (Leiper & Maughan, 1988). The results of expt 3 follow the expected pattern: when the rate of delivery of a labelled solution of constant composition to the small intestine is also constant, the rate of appearance in the circulation of the added tracer is closely related to the tracer concentration.

In most of the experimental situations where the deuterium tracer method has been used, solutions of different composition have been compared. The test drinks chosen for these studies, including those of Davis et al. (1987) and Rehrer et al. (1992), were known to be different in their rates both of gastric emptying and of intestinal absorption. Indeed, the purpose of using this method, rather than measuring either of those processes separately, is to determine the integrated response. Rehrer et al. (1992) measured gastric emptying and blood deuterium accumulation rates simultaneously after ingestion of drinks of different composition, and found a reasonably good \( r = 0.627; P < 0.01 \) agreement between the measured rate of gastric emptying and the rate of \(^2\text{H}\) accumulation in the blood. There was not good agreement between the rate of blood deuterium accumulation for the different drinks and the measured rates of net water absorption in the small intestine, but \(^2\text{H}\) uptake was measured during exercise and intestinal absorption was measured at rest on a separate occasion.

In spite of the evidence to show that the tracer accumulation method produces results that are in broad agreement with predictions made on the basis of the known gastric emptying and intestinal water absorption characteristics of the test drinks compared, doubts have been raised as to the usefulness of this technique (Gisolfi et al. 1990). In the study of Gisolfi et al. (1990), the net rate of water flux in a segment of the small intestine was measured using a triple lumen perfusion method while three different solutions were perfused at a fixed rate; the test solutions were distilled water, a 6% carbohydrate-electrolyte solution and a 10% glucose solution. Deuterium was added as a tracer to all the test solutions. No difference between the different solutions in the rate of blood \(^2\text{H}\) accumulation was found, although there were, as expected, differences between solutions in the rate of net water absorption measured using the perfusion method: a net secretion of water flux in this segment of the intestine was promoted by the
concentrated glucose solution, but there was nevertheless an increase in the blood tracer concentration. The tracer method, of course, is a measure of unidirectional water flux only, and water can move freely in both directions across the mucosal wall. The bidirectional fluxes of water are large relative to the net absorption rate and suggest that minor changes in either influx or efflux rates can result in considerable changes in net transport. The authors concluded that the rate of appearance of a $^2$H tracer added to solutions present in the small intestine does not give a reliable measure of the net water movement.

These results, however, do not provide adequate grounds for rejecting the use of this method as it has been applied in other studies. In reply to a query regarding this study the authors indicated that while unidirectional flux of deuterium out of the jejunal lumen occurred from all three solutions, the relative flux rates followed a pattern which was consistent with the measured net water movement from these solutions (Gisolfi et al. 1992). It is possible that the flux of deuterium from such a large, constant supply of tracer masked the difference which can be detected in the blood accumulation rate when a single bolus of labelled drink is consumed. The use of a continuous infusion into a short segment of the small intestine of a test solution with a constant tracer concentration does not reflect the situation that exists after ingestion of a single bolus of a test drink of the same composition. Solute and water fluxes will vary greatly along the length of the small intestine, and the short (40 cm in the study of Gisolfi et al. 1990) segment through which the test solution is perfused may not be representative of the situation that exists when the whole length of the gut is available for exchange (Rolston et al. 1990). After ingestion of a single bolus of a labelled solution, the concentration gradient from lumen to mucosa will decrease as the tracer is absorbed. The composition of the luminal contents, with regard to the content of actively absorbed solutes and electrolytes, will also change over time, but the perfusion method does not allow for the effects of these changes to influence the measured absorption rates: fresh perfusate is added at the top end of the segment perfused and unabsorbed solution is removed at the end of the test segment. Given these constraints on the interpretation of data obtained using the perfusion method, the criticisms of the technique implied by the results of this study may not be valid.

In contrast to the results of Gisolfi et al. (1990) and Rehrer et al. (1992), there are data to show that there is good agreement between the results of perfusion studies and those obtained with the deuterium tracer method. Leiper et al. (1988a) have reported that the rate of deuterium accumulation was about 50% faster after ingestion of a dilute carbohydrate–electrolyte solution than after ingestion of plain water: this same solution was shown to slightly slow the rate of gastric emptying (Leiper et al. 1988b) but to promote an increase in the rate of intestinal water uptake (Leiper & Maughan, 1986).

REFERENCES


