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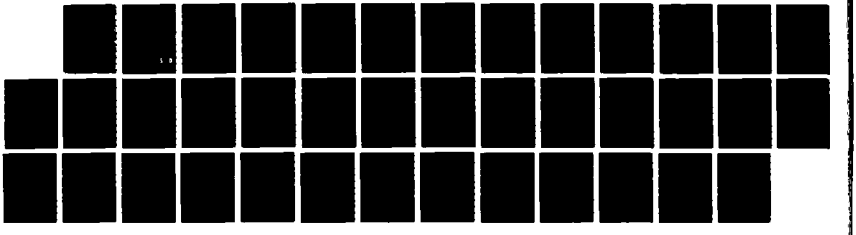

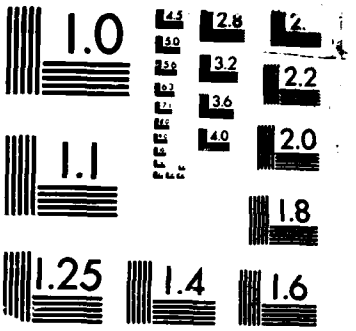

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STABLE ISOTOPE TECHNOLOGY APPLIED TO CONTROLLED DEHYDRATION, RATF OF ENTRY, AND WATER TURNOVER STUDIES IN HUMANS

FINAL REPORT

MORTEZA JANGHORBANI, Ph.D.

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Three experiments were conducted in human subjects: (Water)

1. Investigation of sampling protocols for optimum sampling in studies using H_2O as metabolic tracer.
2. An experiment designed to obtain initial data on changes in Total Body Water (TBW) consequent to experimental dehydration and to define conditions for sequential measurement of TBW.

and 3. An initial experiment addressing the relationship between rate of absorption of water and composition of drinking solution.

It was found that use of mixed saliva did not permit accurate sampling due to problems of contamination with exogenous water, while a special device (Curby cup) was very effective for collection of parotoid saliva. While not effective

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19. For studies of rate of absorption of water, urine was a suitable sampling medium for measurement of TBW.

Sequential measurement of TBW required use of special correction procedure for drifting baseline. This was found to be an important experimental problem in relation to accurate measurement of small decrements in TBW.

Data obtained indicated that rate of absorption of water from drinks containing significant concentrations of carbohydrate/electrolytes did not differ consistently from that for pure water.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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PI Signature

Sept 1987
Date

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SUMMARY

Three experiments were conducted with human subjects with the overall objectives of developing the method of $H_2{}^{18}O$-tracer for studies of water metabolism in relation to heat research. These were:

1. An experiment to define the optimum sampling procedures.
2. An experiment to investigate the significance of composition of drink in relation to observed rate of absorption of water.

and

3. An experiment designed to begin exploration of decrements in Total Body Water (TBW) consequent to mild experimental dehydration

Detailed data are given defining the changes in the ratio $^{18}O/^{16}O$ in various body fluids over the entire length of time from immediate post ingestion of $H_2{}^{18}O$ to 7 hours. Data are presented for plasma, red cells, urine, mixed sweat, mixed saliva, and parotid saliva. It is concluded that, while the method may be suitable for studies related to both Total Body Water (TBW) and the initial rates of appearance of the ingested label in peripheral circulation, proper sampling is an important requirement for each specific experiment.

Data from the experiments focusing on rate of absorption of $H_2{}^{18}O$ show a large intra-and inter-individual variability compared to any effect that composition of drink might have on this parameter of availability of drinking water.

And the results from the controlled dehydration experiment indicate that decrements in TBW can be followed during mild dehydration experiments. However, for accurate measurement of such small changes in TBW suitable correction procedures need to be formulated to account for the observed changes in the isotope ratio for $^{18}O/^{16}O$ during succeeding dosing protocols.

INTRODUCTION

This constitutes the final report for research conducted under the Contract No. DAMD17-86-C-6167, for the period 3-1-86 through 2-28-87.

The overall objectives of the work were to show the feasibility of use of the $H_2{}^{18}O$-method for the accurate measurement of Total Body Water and studies related to the Rate of Absorption of Water, in military recruits.

Three experiments were performed in the Environmental Chambers of USARIEM in collaboration with the Heat Research Division. These experiments were concerned with: a) Sampling Procedures, b) Controlled Dehydration, and c) Rate of Absorption of Water. The subjects who participated in these experiments were recruited from the subject pool available at USARIEM, the Staff of USARIEM, or research staff of Boston University. The protocols were approved by the relevant committees both at USARIEM and Boston University School of Medicine. Each subject signed an informed consent.

EXPERIMENT I- Sampling Procedures

Previous investigations have established the sampling procedures for this method in relation to the measurement of Total Body Water (TBW) (1,2). These procedures involve: administration of a single dose of $H_2{}^{18}O$ in the fasting state, followed by collection of blood/urine at times 2-3 hours after dosing. Several studies of potential interest to heat research involve the need for sampling during the early phase of absorption (EXP. III), or under the conditions where fluid intake could not be limited. Therefore, it was necessary for us to re-examine the issue of sampling procedures under the conditions relevant to studies of heat research. This experiment was designed to investigate the sampling protocols for investigations involving *ad libitum* availability of drinking water. The issues related to sampling under the conditions of fasting will be discussed under EXP. III.

A. Protocol. Four adult males, in apparent good health (Table I), participated in this experiment. Each subject entered the environmental chamber (WBGT=91.7±0.1° F, Rel. Humidity=67%) between 0730 and 0800. Each subject was instructed to consume a light breakfast about two hours prior to entering the chamber. Upon entering the environmental chamber, the subject was fitted with an intravenous catheter, a rectal probe, and heart rate monitor. Each subject exercised on a bicycle ergometer (Monark Model 868, Monark Co., Stockholm, Sweden) for about 15 min (age-adjusted max. heart

Table I- Characteristics of the Participants in Experiment I

Subject Code	Age (yrs.)	Weight (kg)	Surface Area (m2)	Height (cm)
MJ	43	58.8	1.67	178
LA		66.8	1.84	166
RH	47	88.1	1.96	168
GT		94.3	2.14	180

Table II- TBW Calculated from Different Body Fluids
(data are for MJ)

Time, min.	TBW, grams[1]		
	Plasma	RBC	Mixed Saliva
120	34651	34691	35376
180	35268	35120	-
242	35977	36385	36625
304	36831	37021	37576
363	37296	37604	38479

[1]mean difference between RBC and plasma values is 0.6%.
mean difference between mixed saliva and plasma values is 2.3%.

rate 53-69%). At this time, baseline samples of blood (5ml), mixed saliva (2-5ml), sweat(2-5ml), and urine were obtained. A nasogastric tube (Entriflex, 109 cm, 8 Fr., Biosearch Medical Products, Inc., Somerville, N.J. 08876) was passed into the stomach (approx. 75 cm) and 100.0 ml of $H_2{}^{18}O$ was administered followed by an equal amount of tap water.

Samples of blood, mixed saliva, sweat, and urine (2ml) were obtained, as close to 10-15 min intervals as possible during the initial hour after dosing; thereafter, at hourly intervals for the following six hours. Blood samples were drawn in heparinized tubes, centrifuged as soon as possible, and both plasma and red cell fractions saved for isotopic analysis. At the termination of this phase of the experiment, samples were frozen (-20° C) until analyzed.

In two of the subjects, Curby cups (2) were also inserted, but only after the initial hour postdosing. This was done to investigate the effect of exogenous contamination on saliva samples. At each sampling time, the Curby cup was inserted after collection of mixed saliva, but as soon thereafter as possible (within a few minutes). Saliva flow was stimulated with a Lemon Drop, and about 2-3 ml saliva was collected.

Subjects were allowed to drink *ad libitum* (Sunkist Light, Thomas Lipton, Inc., Englewood Cliffs, NJ), but records were kept of their fluid intake. At noon, a light lunch--a sandwich, a can of soda, and a small bag of potato chips--was served. No attempt was made to serve the same lunch to all subjects or to regulate their intake.

B. Appearance of Label in Various Body Fluids. The course of appearance of the label in various body fluids is shown in Fig. 1 for all four subjects. As expected, the isotope ratio rises rapidly during the initial 25 min after ingestion of the label. The magnitude of isotope ratio was practically identical for samples of plasma and the corresponding red cells. This was true for both subjects whose red cells were analyzed (for two subjects, red cells were not analyzed). Peak plasma appearance occured at 20-30 min after ingestion of the dose for all four subjects. Thereafter, dilution from the vascular space into interstitial water/intracellular water resulted in a sharp decline in the observed isotope ratio for plasma up to about 75-100 min, depending on the subject. Following the initial two hours postdosing, the isotope ratio in blood declined gradually, but consistently for the remainder of the eight-hour observation period. This was the case for all the four subjects. The subjects were allowed water *ad libitum* so that there was dilution from exogenous water. Nevertheless, the possibility of incomplete equilibrium with total body water cannot be dismissed.

Important differences were observed among various body fluids in regard to their isotope ratios. Below, we discuss the observed changes for each fluid in comparison with blood.

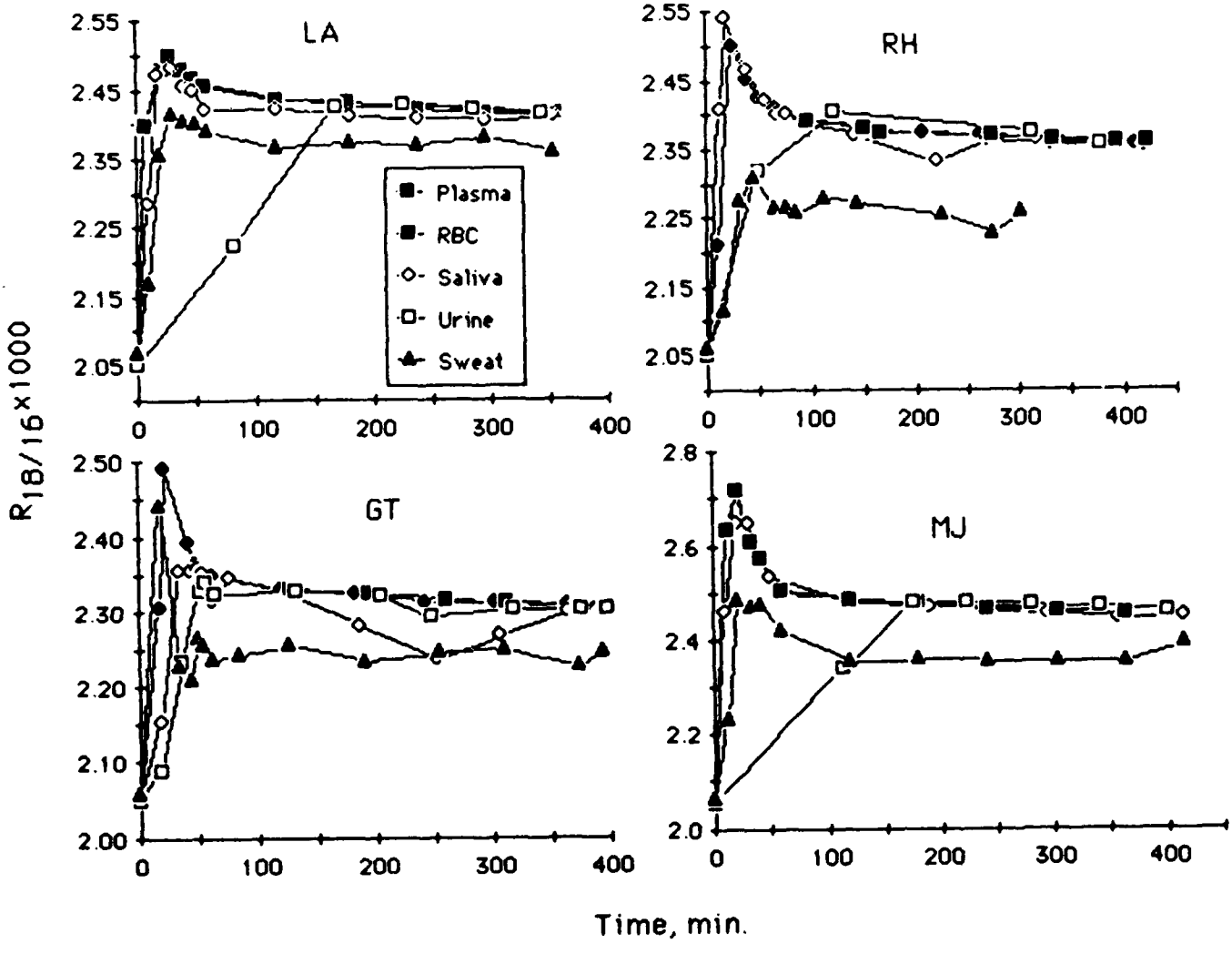

Fig. 1- Dynamics of Appearance of Labeled Water

1. Plasma vs. Saliva. The appearance curves of mixed saliva and the corresponding plasma for the initial 60-min. postdosing period are shown in Fig. 2. While the general pattern between plasma and mixed saliva curves is similar, quantitatively significant differences are observed. In three of the subjects, plasma was more highly enriched than saliva, at least in some of the samples. This may be an artifact of the sampling procedure as saliva samples reflect the integral of any sampling interval. In contrast, plasma samples should represent the end-point of the interval. In one subject the situation was reversed. In all four subjects, the isotope ratio between plasma and mixed saliva became quantitatively the same by 40-min postdosing, at the latest. This reflects the quantitatively insignificant role of gastrointestinal absorption of water after this time.

The data presented in Fig. 2 were obtained when the labeled water was administered through a nasogastric tube. This was done to avoid contamination of the oral cavity with the label. However, this procedure suffers from obvious limitations, especially under field conditions.

The comparative data between mixed saliva and plasma for the time period after the initial hour have been plotted in Fig. 3. For both MJ and LA, the saliva appearance curves show a somewhat lower isotope ratio than the corresponding plasma curves. The differences are larger than can be attributed to any systematic errors of measurement. The difference may reflect slight contamination from exogenous water present in the oral cavity. It could also have resulted from preferential secretion of the lighter isotope. That the former cause is perhaps more important is apparent from consideration of the mixed saliva in comparison with the Curby cup collections for the other two subjects (Curby cup collections were not made for the former two subjects). The mixed saliva samples for both RH and GT show the slight reduction in isotope enrichment observed for the former two subjects. However, they also show large excursions downward followed by a trend towards the plasma isotope ratio. The observed large excursions correspond to intake of drinks and are clearly consequences of dilution with exogenous water. For the latter two subjects for whom Curby cup collections were available, all data show excellent correspondence with their respective plasma data (also see section under TBW for quantitative comparisons). The data clearly point out the potential contamination problem inherent in mixed saliva. Even in the absence of gross contamination due to a drink, that from exogenous water during sample collection may not be negligible. These observations lead to the conclusion that mixed saliva collected in the manner employed in these experiments is not suitable for accurate isotopic studies.

2. Plasma vs. Sweat. The general pattern for appearance of the isotope in sweat as compared with other body fluids is shown in Fig. 1. Two observations become evident from comparing the

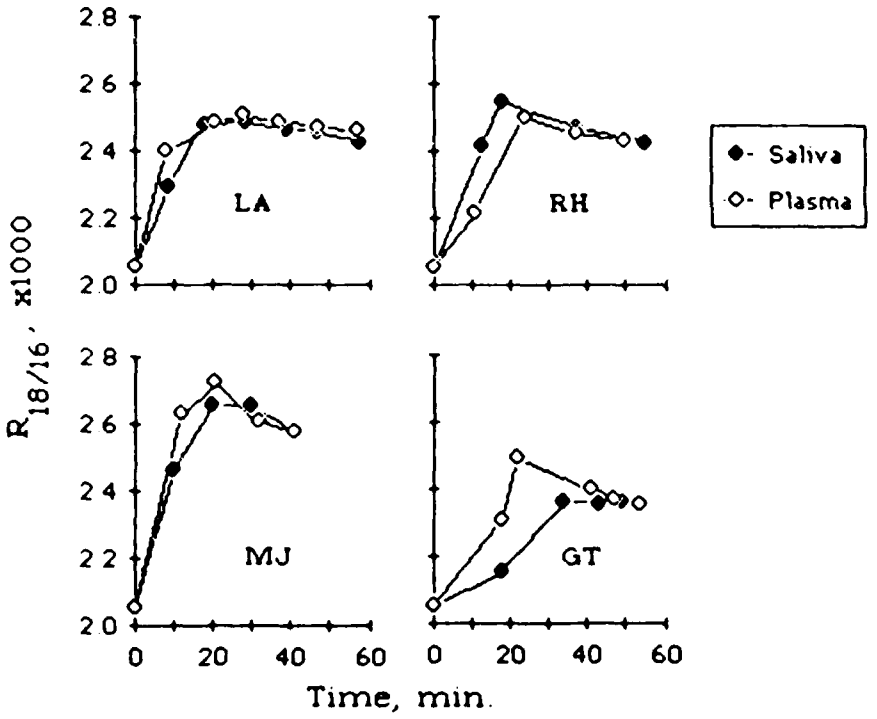

Fig. 2- Early Phase of Isotope Appearance in Plasma and Mixed Saliva

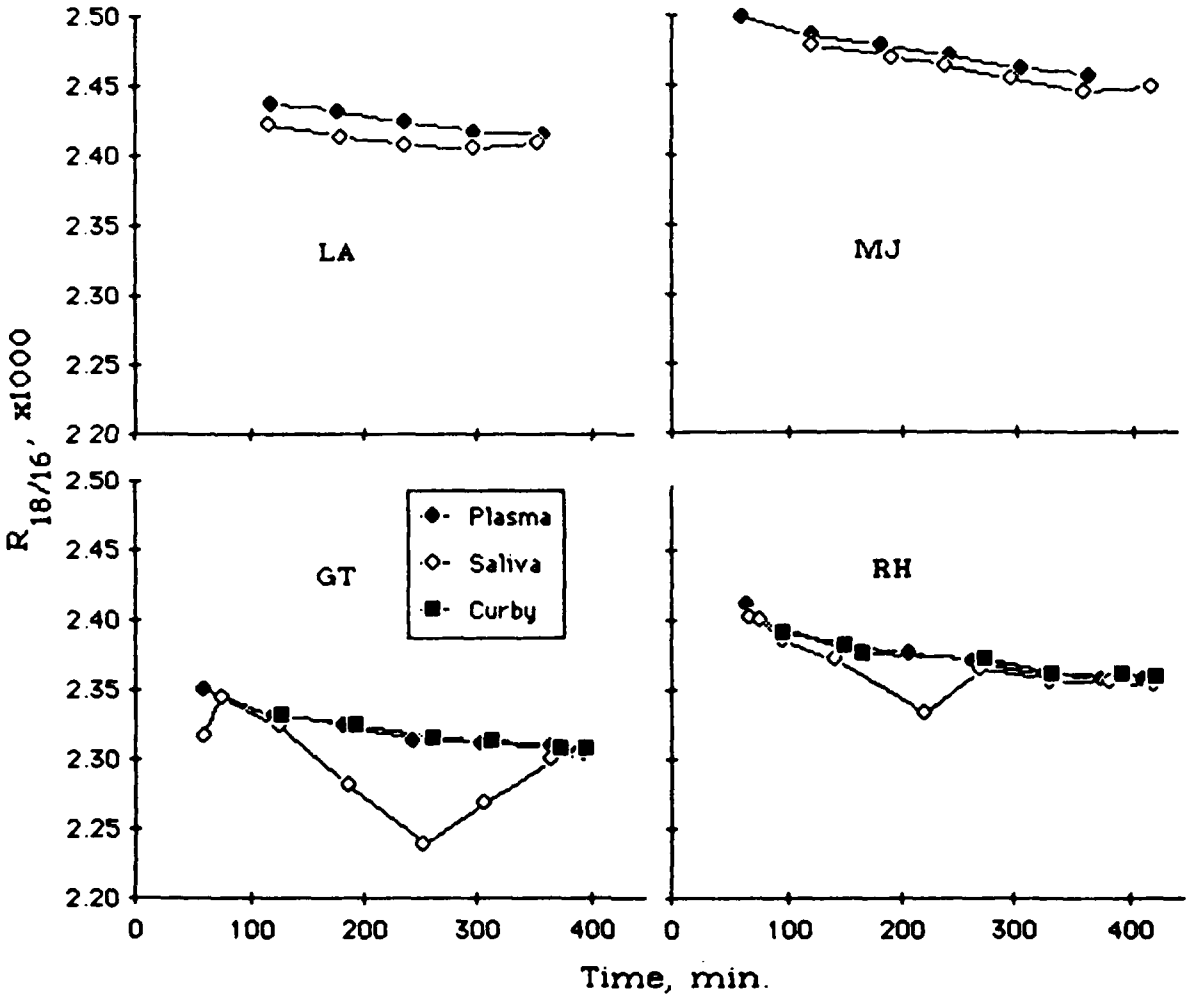

Fig. 3 - Late Phase of Isotope Appearance

appearance curves for sweat with those for plasma. First, isotopic enrichment in sweat was always substantially less than for plasma. And secondly, evidence of contamination was present randomly throughout the sweat appearance curves. These data were obtained under the conditions of high humidity (RH=65%). No attempt was made to prevent dilution of sweat with exogenous water. This was done to evaluate the extent of such contamination in light of the possible use of sweat for such studies. From these data it is concluded that sweat sampling in this manner is not suitable as a technique for accurate assessment of the dynamics of water transport. This does not indicate total lack of value for sweat samples in such studies, however. Uncontaminated sweat can be collected reliably employing arm-band techniques.

A second practical problem in the use of sweat samples is related to the availability of sweat. Even under the conditions of high humidity employed in these experiments, frequent sampling was not always possible. Localized sampling would be very difficult, unless suitable enclosures (e.g. arm bags) are employed. Additionally, any quantitative effect that dehydration might have on the production of sweat remains yet to be explored.

3. Plasma vs. Urine. Urine is a convenient sampling medium for studies of body water. It does not pose problems related to exogenous contamination. However, it should not be expected to permit studies of the early phases of water absorption. This is evident from the data shown in Fig. 1. Urine samples obtained after two hours postdosing showed evidence of equilibrium with systemic circulation. Thereafter, isotope ratios were very similar for the two body fluids.

The data show an initial phase during which urine isotope ratio increased towards that for plasma. This increase appeared to be almost linear function of time. In this study, the bladder was emptied prior to ingestion of the label, but thereafter water intake and bladder evacuations were carried out *ad libitum*. This was done to permit evaluation of sampling protocols in relation to future field applications of the technique.

Therefore, these data do not permit evaluation of the earliest time corresponding to establishment of isotopic equilibrium between urine and that for plasma. However, they do show that from a practical point of view equilibrium was achieved by about 200 minutes. Thereafter, the isotope ratios were practically identical for the two matrices.

C. Calculations of Total Body Water (TBW). Total Body Water was calculated for each subject as a function of time, starting with 100 minutes postingestion except for urine for MJ and LA for whom the earliest time was taken at 180 minutes (Fig. 1). The caculations were carried out according to the following equation:

$$^{16}O_{TBW} = [\ ^{18}O^*_r] \ / \ [\ R-R^\circ] \qquad (1)$$

$^{16}O_{TBW}$: oxygen-16 content of TBW, g-atoms

$^{18}O^*_r$: oxygen-18 from the ingested label, retained at the time of sampling

R : isotope ratio (g-atom basis) in sample

R°: isotope ratio (g-atom basis) in the baseline sample

These calculations are exact except for two simplifying assumptions. First, the quantity of $^{18}O^*_r$ is taken equal to the amount ingested. This is an accepted practice (1,2), as both unabsorbed label and its loss via various pathways prior to establishment of equilibrium are negligible. After the isotopic equilibrium has been established, loss of the label does not alter the ratio in the body water space unless entry of water is significant. The second simplifying assumption relates to neglecting the oxygen-16 content of the labeled water. Compared with the oxygen-16 content of the body water, this is certainly negligible. Total Body Water (grams) is then calculated as $18.0152x[^{16}O_{TBW}]$.

1. Calculations of TBW Using Different Body Fluids. The isotope ratio data given in Fig. 1 show the expected correspondence in the calculated value of TBW employing different body fluids. Based on the general trends observed, it should follow that sweat samples should not be expected to provide useful data for these calculations, under the conditions of collection employed here. Mixed saliva samples will also overestimate the true values of TBW, due to inevitable dilution from exogenous sources. Whether urine will be suitable for this purpose depends strongly on the frequency of voiding.

Data summarized in Table II show the actual magnitude of the correspondence in the calculated value of TBW among plasma, RBC, and mixed saliva for MJ. This subject's mixed saliva samples showed the closest agreement with plasma, among the four subjects. The data clearly demonstrate the very close agreement between plasma and RBC (mean difference 0.6% of the mean value), but also that even for the best set of data, TBW calculated from mixed saliva could be in error by 2-3%, a potentially serious error (Table II). That the Curby cup method provided suitable sampling medium, compared with plasma, is shown in Figs. 3 and 4 for the two subjects GT and RH. In this experiment, water intake of the two subjects was both *ad libitum* and large. Therefore, a significant component of the observed expanding pool is related to this. Direct

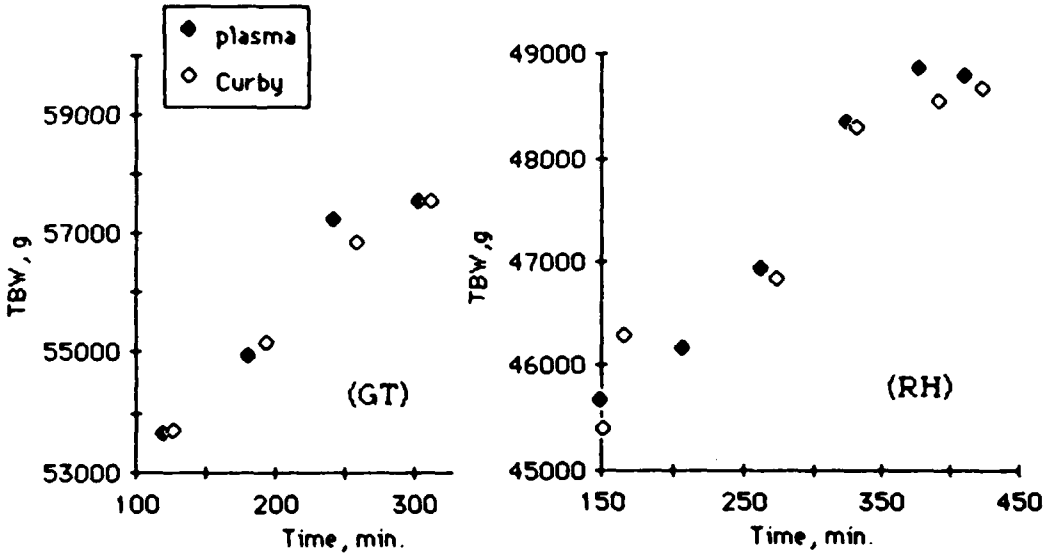

Fig. 4 - Correspondence in the Values of TBW between Plasma and Parotid Saliva for two Subjects

comparison between the values of TBW calculated from plasma and saliva (Curby cup) for these two subjects is not possible due to the unavoidable differences in sampling times. However, the least-squares regression equations for each set of data (Table III) clearly demostrate the equivalency of the two sampling media. Based on the regression parameters of the two sets of data for each subject we can readily determine the maximum divergence of TBW calculations using either medium. These calculations (Table III) show clearly that in no case was the difference greater than 0.7% of the mean value. This corresponds to a maximum divergence of 343 g.

2. Correlation of Total Body Water (TBW) with Body Weight. We have summarized our data for the four subjects in Table IV. The data clearly show excellent correlation for our subjects. The coefficient of correlation (r) for these four subjects was 0.96; excluding RH, it was 0.99. While the small number of observations in this study preclude generalized statements, the data present much higher coefficient of correlation than given by Moore, *et al* (4) for their normal series. The ratio of TBW to BW in the present study was in the range 0.51-0.59 (Table IV) and decreased with increasing Body Weight. The range for the corresponding ratio in Moore's normal male series was 0.46-0.60 (4). It is interesting to note that if the ratio of actual body weight to the desirable body weight is calculated for these four subjects (Table 1-3, ref. 4), the following values are obtained: MJ 0.89, LA 1.09, GT 1.15, and RH 1.38. Therefore, the observed decrease in the ratio of TBW/BW appears to be related to increasing body fat content, especially for RH. It would then follow that if TBW were plotted against Lean Body Mass (LBM), even a stronger correlation would have resulted for our subjects, as is expected (4).

D. Conclusions. This study was initiated to investigate the methodological issues related to proper design of experiments based on the $H_2{}^{18}O$ stable isotope tracer method, for specific application to issues of water transport in studies of dehydration. Two general components of these applications were addressed specifically:

1) Experiments aimed at studies of Rate of Absorption of Water. These experiments require frequent sampling during the initial hour after administration of the label.

2) Studies related to accurate measurement of Total Body Water (TBW) and its possible alterations during the course of dehydration-rehydration. These experiments require very precise measurement of the isotope ratios and the administration of optimum dose of $H_2{}^{18}O$.

Based on the experimental findings of this study, the following conclusions are offered:

Table III- Least-Squares Regression Parameters for TBW on Time[1]

Subject		a	b	r^2
R.H.	Plasma Data	43548	13.507	0.961
	Curby Cup Data	43981	11.530	0.934
G.T.	Plasma Data	51600	19.46	0.951
	Curby Cup Data	51489	19.05	0.983

[1]TBW= a+ b.t
TBW in grams
Time in minutes

Table IV- Data on Total Body Water vs. Body Weight

Subject	Body Weight, g	Total Body Water, g[1]	Ratio
MJ	58820	34670	0.59
LA	66760	39100	0.59
RH	88049	45270	0.51
GT	94250	53860	0.57

[1]these measurements refer to TBW at 120 min. postdosing
TBW= 6872 + 0.472(BW), r=0.96

1) The required precision (RSD) for the ratio $^{18}O/^{16}O$ depends on the dose level and the specific purpose of the experiment. It should be 0.01% or letter.

2) The stable isotope calibration method used to convert per mil values to g-atom ratio for $^{18}O/^{16}O$ appears to be independent of any matrix effects. Thus, it is sufficient to employ water as the matrix for preparing spiked standards.

3) The required dose level of $H_2{}^{18}O$ depends on the nature of the experiment, the desired accuracy, and the expected magnitude of changes in TBW. For a 70-kg reference male, 10-100 ml of a nominal 10% $H_2{}^{18}O$ costitutes the optimum dose.

4) The best sampling medium (for field studies) is parotid saliva collected by means of a suitably constructed Curby cup. Sweat samples are not suitable unless collected with attention to exogenous contamination. Urine samples may be suitable for studies of TBW, but require attention to bladder voiding.

5) Studies designed to investigate changes in TBW require careful control of sampling time. Complete mixing of the absorbed label with TBW is not achieved after 2-3 hours postdosing. This issue requires further resolution in the context of the expected small decrements.

6) In a properly designed experiment, changes in TBW of a few hundred grams should be measurable. In a 70-kg reference man, this amouonts to fraction of one percent of TBW.

7) This method should be well suited to field investigations of TBW in exercising individuals.

EXPERIMENT II- Rate of Absorption of Water

Introduction. It has been suggested previously (5) that one of the reasons for desirability of including salts/carbohydrates in the drinking solution is related to improvements in the rate of absorption of water. Since the cost of providing troops with such formulations is much higher than water, it is important to invesitgate the scientific basis for the claim.

A. Protocol. Six subjects participated in a two-day experiment designed to investigate rate of absorption of $H_2{}^{18}O$ consumed with or without Gatorade. On day 1, subjects engaged in sedentary activities under thermoneutral conditions. On day 2, they engaged in exercise under hot conditions. Each subject drank 450 ml of solution containing either D.I. water or Gatorade over a 1-2 min. period and spiked with $H_2{}^{18}O$. The test was repeated twice on each of the two days. On each test day the subjects drank both solutions randomized in sequence.

B. Sampling Prcedure and Calculations. Plasma Samples were collected over the 60-120 min. period following each drink. Each sample was analyzed for its excess ^{18}O (compared with that present in natural water) resulting from the appearance of the ingested label. Isotope excess data were converted to mole ratio data for $^{18}O/^{16}O$ by use of isotope calibration standards. The following expressions were used to calculate absorption of $H_2{}^{18}O$ as a function of time:

$$R_{18/16} = R°_{18/16} + [(^{18}O^*_{abs.})/(^{16}O)] \qquad (1)$$

$$[\%abs.]_t = [^{18}O^*_{abs.}/^{18}O_{admin.}]_t = 100x(R_t - R°)x(^{16}O_{TBW})/(^{18}O_{admin.}) \qquad (2)$$

Calculations of ^{16}O of total body water ($^{16}O_{TBW}$) were based on the measured value of TBW from experiments on Controlled Dehydration, as given in Table V.

C. Assumptions. To convert the isotope excess data to %abs., we have assumed that there is instantaneous mixing of the absorbed label with the total body water. This is obviously an incorrect assumption which will lead to artificially high values for %abs. during times prior to about 120 min. However, since the purpose of these studies is to compare the effect of oral solution formulation on the rate of absorption and its equilibrium value, the error of this assumption is not of any consequence.

D. Observations. The data of this experiment have been summarized in Fig. 5. Based on these data, the following conclusions have been reached:

1- For each subject, the rate of entry of the label in either plasma appears to be more variable than any consistent differences resulting from the composition of the drinks. This could be due to a number of methodological/physiological reasons: for plasma samples, frequency of sampling could be the limiting factor, or the intraindividual variability in the rate of appearance of the ingested label could be considerable.

2- For all subjects and test periods, absorption of the label appears to be complete within the initial 30-40 minutes. However, for each subject the individual rate-of-entry curves vary considerably during the intial 30-40 min. time period.

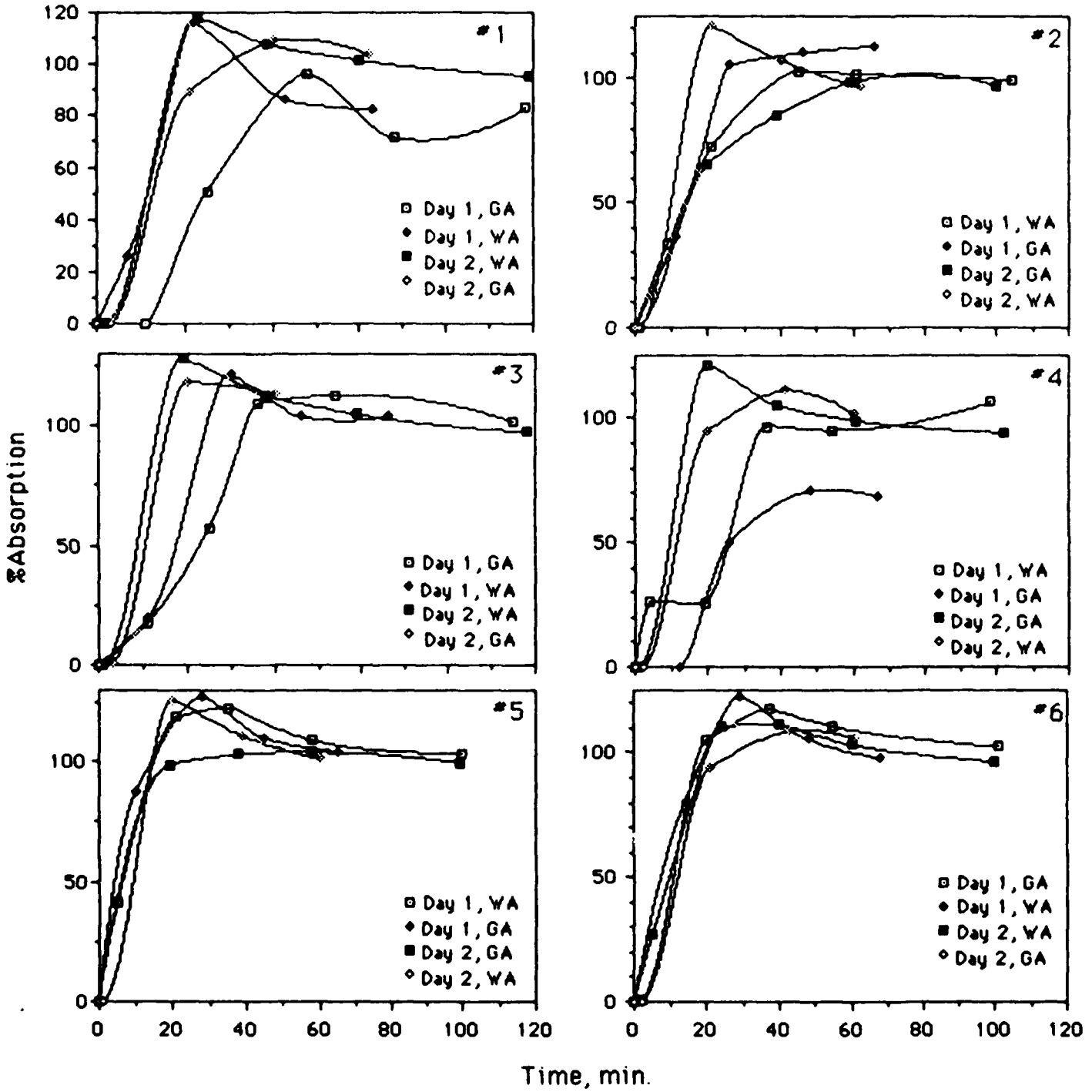

Fig. 5- Rate of Absorption of Labeled Water in Six Adults

3- In general, the rate of entry of the label drank with the Gatorade solution on the day of exercise may be more drawn out than the corresponding water drink or the drinks for the rest day This is most clearly observed for subject #2, subject #5, or subject #6.

4- For three subjects, the rate-of-absorption curve for the Gatorade solution suggests significant delay or incomplete absorption of the label (subject #4 day 1, plasma curve; subject #1 day 2; subject #3 day 2). These may be artifacts of the method, rather than reproducible physiologic observations.

E. Tentative Conclusions. There does not appear to be a consistent difference in the initial rate of absorption of water related to the composition of the drinking solution. However, the initial rate of the individual curves appears to vary considerably. In general, absorption of the drink appears to be complete within the initial 30-40 min. The shape of the curve varies markedly in some individuals, but not others. The overall observed differences could be very marked.

Based on this study, one cannot draw definitive conclusions about the effect of solution composition on the rate of absorption of water. This is primarily due to the observed intraindividual variability of the curves. Thus, if the magnitude of the observed changes is deemed important in relation to the availability of the drink, one needs to investigate under what conditions, if any, the intraindividual variability could be reduced.

EXPERIMENT III- Controlled Dehydration

An initial experiment was carried out to determine the accuracy of sequential measurements of Total Body Water in the context of the expectedly small decrements occuring during controlled dehydration in single-day experiments.

Introduction. A progressive controlled dehydration experiment was designed with six adult subjects to investigate the relationship between Total Body Water (TBW) as determined with the $H_2{}^{18}O$-method and Body Weight (BW), as well as the changes brought about in these two parameters due to experimental dehydration.

A. Protocol. The study was carried out on four days over a period of two weeks. On each test day, the subjects reported to the environmental chamber kitchen facility at about 0700. They were given an instant breakfast and generous amount of water to maintain a high state of hydration. At about 0800 they entered the environmetal chamber maintained under thermoneutral conditions. They were given a solution of $H_2{}^{18}O$ (30-35 g at 18% ^{18}O) to drink followed by a 50-ml rinse water. At 1500 hours, they repeated the

dosing procedure. At 1800-1830 they were dismissed. Except for the test water (160-170 ml), they remained in a complete fasting state for the duration of each test day (10-11 hours).

Progressive dehydration was induced in the subjects via fasting combined with exposure to high temperature of a second environmental chamber in which they stayed for periods of 0-5 hours (day 1, 0 hrs; day 2, 1 hr; day 3, 3 hrs; day 4, 5 hrs) prior to their second test drink. The remainder of the entire test day was spent in the chamber maintained under thermoneutral conditions.

Blood and parotid saliva (Curby cup) were collected prior to each test dose, at two and three hours after each test dose, and at hourly intervals prior to ingestion of the second test dose.

B. Analyses. Each sample of saliva and plasma was analyzed for ^{18}O-enrichment. The ^{18}O-excess data were converted to $^{18}O/^{16}O$ ratios (g-atom basis) via a calibration procedure employing deionized water spiked accuratey with known increments of the $H_2{}^{18}O$ used in the human experiments. In this way, any systematic errors were avoided. The resultant data were in the form of the ratio of $^{18}O/^{16}O$ expressed on g-atom basis.

C. Calculations. Total Body Water (TBW) was calculated from the following expression:

$$TBW\ (grams) = 18.0152 \times (^{18}O^*_r)/(R-R^*) \qquad Eq.\ (1)$$

TBW= Total Body Water, g

$^{18}O^*_r$= g-atoms of ^{18}O administered, assumed completely absorbed and retained

R= g-atom ratio of $^{18}O/^{16}O$ after mixing has been completed (between 120-420 min. after dosing)

R^*= g-atom ratio of $^{18}O/^{16}O$ in the baseline sample, corrected for isotope decay

The constant 18.0152 corresponds to the molecular weight of water, of natural composition.

D. Results and Discussion.

1- Reproducibility and Accuracy of Isotopic Measurements. Calibration standards were analyzed on five occasions during the course of these analyses. The results have been summarized in Table VI.

The calibration data show both a high degree of reproducibility as well as linearity. The Relative Standard Deviation (RSD) of the analyses carried out on the four occasions was in the range ±0.1-0.3 per mil for the five standards. All calibration data were combined to yield the following least-squares linear regression equation:

$$\text{Delta (per mil)} = -1037.3 + 504{,}119.2 \times R \qquad (r^2 = 0.999986)$$

2- Reproducibility of Isotope Ratio Measurements between Plasma and Saliva Samples. Typical data comparing the correspondence between the plasma and saliva isotope data have been summarized in Table VII. These are typical of all data. The correspondence between the isotope data, for any time point, between the two media was in general within 1-2 per mil (not as good as for standards). In some instances, the difference was substantially larger. For example, for sample at 8 hours of Day 1 (Table VII), the measured values were 245.8 and 249.8, a difference of 4.0 per mil. This shows an important source of potential error for the determination of small changes in TBW (see later section on implications of this error).

3. Behavior of Isotope Ratios. There was a significant decrease in the isotope ratio during the seven hours after administration of the first dose on each day. This was the case for all subjects and all test days (Figs. 6&7). For example, as shown in Fig. 6 (day two of dehydration exp. for subject #2) the ^{18}O-excess following the first administration of the isotope decreased from 353.9 (120 min.) to 348.7 (420 min.); a change of 5.2 per mil. This unexpected major decrease introduced the potential for a source of error in the accurate measurement of TBW; an important requirement for studies of dehydration in man. The isotope data following the first administration of the label were fitted to least-squares linear regression equations (Figs. 6&7). Highly linear regression plots were obtained (Figs. 6&7).

The rate of decrease appeared to be greater for the successive days of exposure to heat (Table VIII). If this decrease in ^{18}O-excess is truly related to delayed mixing with body water (+any exogenous water entering the body during the period of observation), then there appears to be an enhancement of the body water available for isotopic equilibrium during exposure to heat. This may be an important observation, if it can be confirmed, as it may well indicate a change in mobility of body water as core temperature is increased. When plotted against rectal temperature (Fig. 8), there is a positive correlation. The observed scatter could be explained by the combination of errors in the two measurement parameters so that in a carefully conducted experiment this correlation might be much more significant. Is this enhancement of isotope decay related to the expected increase in cardiac output, the effect of increased core

Table V- Values of Total Body Water used to Determine the Rates of Water Absorption

Subject #	TBW (g)	g-atoms of ^{16}O
1	54892±1771	3040
2	39940±1360	2212
3	41657±1116	2307
4	47562±905	2634
5	48789±334	2702
6	41476±546	2297

Table VI- Reproducibility and Linearity of Calibration Data

$^{18}O/^{16}O$* (g-atoms basis) x1000	Measured ^{18}O-excess** (per mil) (1)	(2)	(3)	(4)	Mean±1S.D.
2.97096	459.7	460.2	460.0	459.5	459.9±0.3
2.50154	224.5	225.0	225.0	225.1	224.9±0.3
2.36633	155.0	155.7	155.4	155.6	155.4±0.3
2.13166	37.1	37.4	37.2	37.1	37.2±0.1
2.04493	-6.9	-6.8	-7.0	-6.9	-6.9±0.1

*based on accurate spiking of deionized water.

**Four independent measurements on separate occasions.

Table VII- Correspondence between Typical Plasma and Saliva Isotope Data

Time, h	Delta, per mil	
	Plasma	Saliva
Day 1, Subj. #1		
0, isotope administered	144.9	144.4
2	197.8	198.1
3	196.7	197.2
4	196.1	196.3
5	195.3	195.8
6	195.6	195.8
7 0, isotope administered	195.2	195.2
8 2	245.8	249.8
9 3	246.1	244.2
Day 2, Subj. #1		
0, isotope administered	177.7	176.6
2	229.5	229.2
3	229.1	228.3
4	228.4	228.8
5	227.7	229.0
6	226.8	228.0
7 0, isotope administered	227.1	226.9
8 2	282.2	283.3
9 3	279.2	282.3

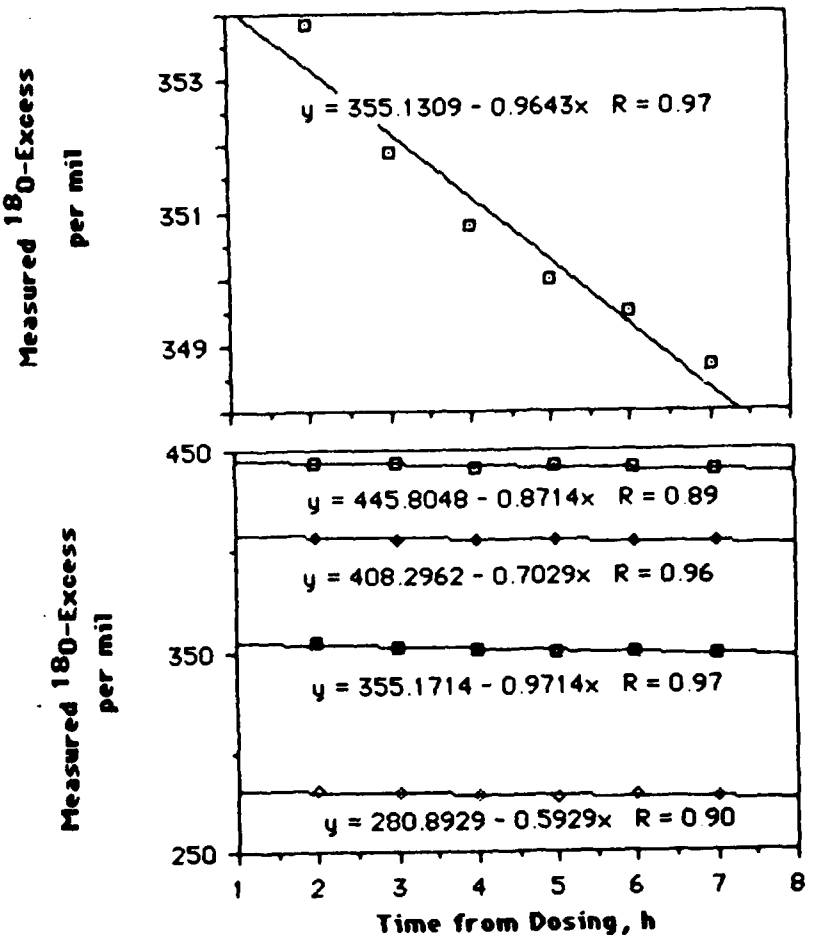

Fig. 6- Course of Isotope Decay for a Typical Period

Fig. 7- Course of Isotope Decay for Sequential Periods

Table VIII- Experimentally Observed Rates of Decrease in ^{18}O-excess

Subject#	Rate of Decrease, per mil/hr			
	CD1	CD2	CD3	CD4
1	0.519	0.452	0.715	1.553
2	0.600	0.977	0.701	0.925
3	0.517	1.049	1.063	0.937
4	0.696	0.446	1.179	1.189
5	0.631	0.866	1.021	0.810
6	0.661	0.720	1.101	1.140
Mean	0.604	0.752	0.963	1.092
1S.E.M.	0.030	0.106	0.084	0.109

Table IX- Calculated Rates of Apparent Expansion of TBW (based on linear regression equations of experimental data)

Subject#	CD1	CD2	CD3	CD4
	(g/hr)			
1	451	534	496	797
2	243	464	363	578
3	388	272	668	644
4	339	192	797	711
5	282	578	735	607
6	312	364	612	688
Mean±1S.E.M.	336	401	612	671
	31	62	65	32

r for the linear regression lines was in the range 0.79-0.99

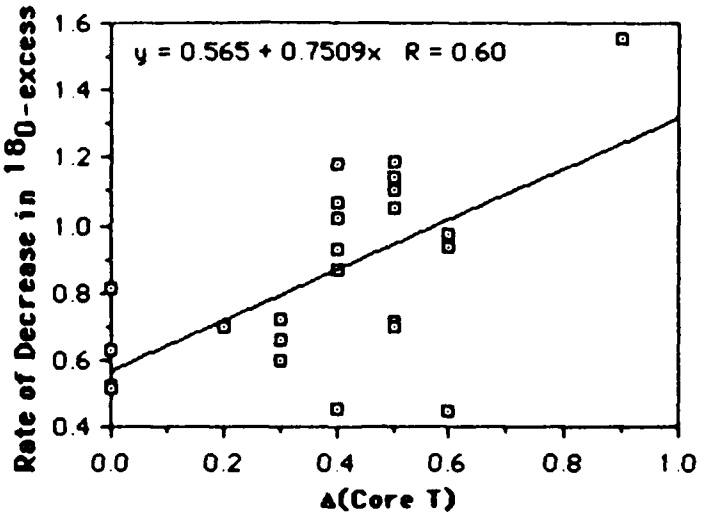

Fig. 8- Effect of Core T on Rate of Baseline Drift

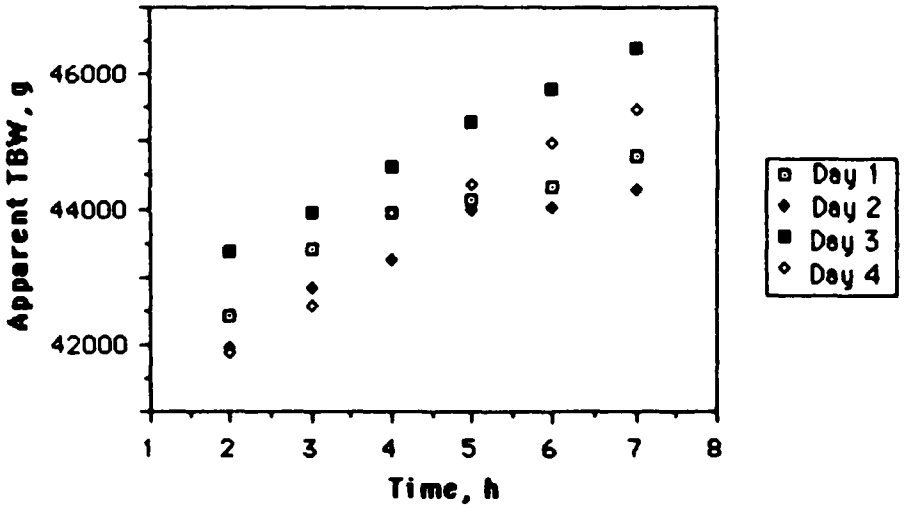

Fig. 9- Plots of Apparent TBW vs. Time for one Subject

temperature on mobility of body water, or a true increase in body water from endogenoous/exogenous sources? Why does the rate of decrease appear to accelerate with increasing exposure to heat? The subjects were fully hydrated prior to start of the experiment. The label was given about 30 min. after breakfast. Is the observed change in the isotope ratio due to some dilutional effect of the large drink that was consumed with breakfast so close to the test dose (an artifact of the experiment)? We assumed that as long as the drink was consumed prior to the label that the label would mix with the drink and endogenous body water within the expected two-hr equilibrium period. This may have contributed to the observed change.

4. Calculation of Total Body Water. Two types of calculations were performed: a) uncorrected TBW, and b) corrected TBW.

a) Uncorrected TBW. The value assigned to R° in Eq. (1) is a critical factor in the correct calculation of TBW. Normally, this corresponds to the natural ratio of $^{18}O/^{16}O$ and is invariant during the course of the experiment. However, in experiments involving multiple dosing, the value of R° also varies in a similar fashion to the value of R. Thus, if we hold the value of R° constant (at the measured value), we will calculate too-large a value for TBW. The calulated value of TBW will increase as a function of time. The increase is related to "new unlabeled water" seen by the isotope. The sources of this "new unlabeled water" could be: true delays in isotopic equilibrium with body water (e.g. cerebrospinal fluid), addition of exogenous water from such sources as air humidity, production of metabolic water (esp. if this is increased in fasting individuals in a hot environment), or water consumed at breakfast.

Typical plots of Apparent Body Water (uncorrected R°) have been given in Fig. 9 for one subject. The rate of expansion has been tabulated for all subjects in Table IX.

The rate of expansion appears to increase with increasing length of exposure to heat. The interindividual variability, as measured by the SEM, is in the range of 5-16%. When the data are expressed in per unit TBW (Table IX), the interindividual variability is reduced to within the range 4-9%, indicating a signficant correlation with TBW.

b) Corrected TBW. Regardless of the reasons for the apparent expansion observed during the postdosing period, accurate sequential assessment of TBW requires application of some correction procedure. We have assumed that the rate of isotope decay observed during the postdosing procedure is the same for both baselines (morning and afternoon baselines, 0800 & 1500). While this assumption could be valid for the afternoon baseline, it may not be exactly accurate for the morning baseline. This is due to the unknown and potentially large intake of water prior to the morning dose.

The slope of the isotope decay regression line (Fig. 6) was calculated for each test period. From this, an appropriate linear decay equation was determined for both baselines. The results of this correction procedure to the calculated value of TBW are shown in Fig. 10 for subject #6(day 4). It should be noted that this is an artificial correction procedure in that the observed apparent expansion is a real observation. But this procedure is necessary if sequential measurements of TBW are to be compared. The present procedure suffers from an incorrect assumption, i.e. equal rate of isotope decay for the two sequential baselines. Because the observed rate of expansion is substantially higher than anticipated from published data, the errors resulting from this assumption are expected to preclude accurate assessment of small changes in TBW. A more appropriate correction procedure is needed for such studies.

5- Observed Correlations. The expected positive correlation between TBW and BW is given in Fig. 11. The coefficient of correlation for linear regression of TBW on BW for our data is R=0.95. The corresponding value given by Moore *et al* for their normal male series (age 15-30) is 0.76 (4). When the data are expressed as the ratio of TBW/BW plotted against BW, the plot of Fig. 12 is obtained. The plot shows the expected inverse relation between these two parameters, in part due to the higher contribution of fat to BW for the heavier subjects.

When the data are expressed as TBW2/TBW1 (TBW after dehydration/ TBW before dehydration) or BW2/BW1 against length of exposure to the hot environment, a potentially important difference between the two sets of data emerges (Fig. 13). The ratio TBW2/TBW1 changes much more than the ratio BW2/W1, as is to be expected. When the data are expressed as mean values for the six subjects (Fig. 13b) the difference becomes dramatic (also see Table XI).

E. Conclusions. The data from this experiment lead to the following conclusions:

1. The proposed method of TBW using $H_2{}^{18}O$ permits investigation of water homeostasis in relation to dehydration in a way not possible with the standard methods based on body weight.
2. The method should permit new insight not only into the dynamics of water homeostasis, but also a much more sensitive approach to the determination of the extent of dehydration.
3. The method is ideal for field applications where non-invasive sampling is required.

F. Recommendations. Two types of issues are raised from the results of this experiment, leading to some recommendations for future use of the method:

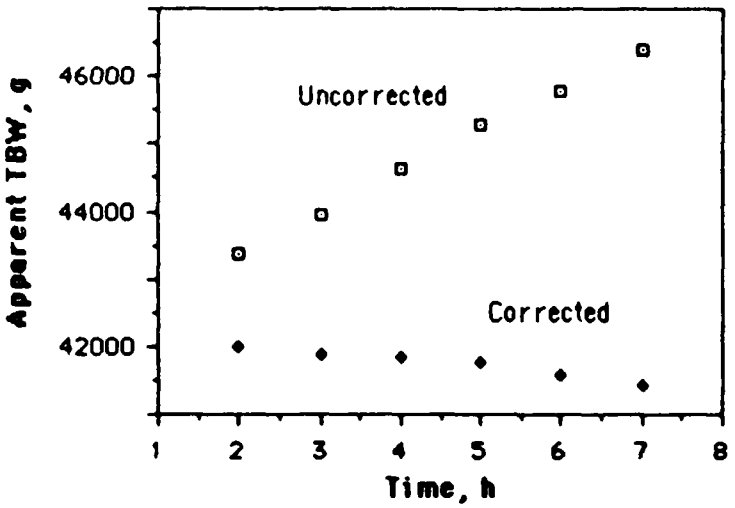

Fig. 10- Effect of Baseline Correction Procedure

Table X- Apparent Rate of Expansion per Unit of TBW

Subject#	CD1	CD2	CD3	CD4
			(g/hr-kg)	
1	8.1	9.5	8.9	15.1
2	6.2	11.9	9.3	13.8
3	9.2	6.8	16.1	15.1
4	7.2	4.0	16.6	15.2
5	5.8	12.0	14.9	12.4
6	7.4	8.8	14.6	17.0
Mean±1SEM	7.3	8.8	13.4	14.8
	.5	.8	.9	.6

Table XI- Effect of Length of Exposure to Heat on Water Loss Parameters

Length of Exposure, hrs.	BW2/BW1	TBW2/TBW1
0	0.986±0.002	0.991±0.009
1	0.984±0.005	0.984±0.002
3	0.981±0.001	0.962±0.006
5	0.973±0.001	0.944±0.010

Data are mean±1S.E.M. of six subjects.

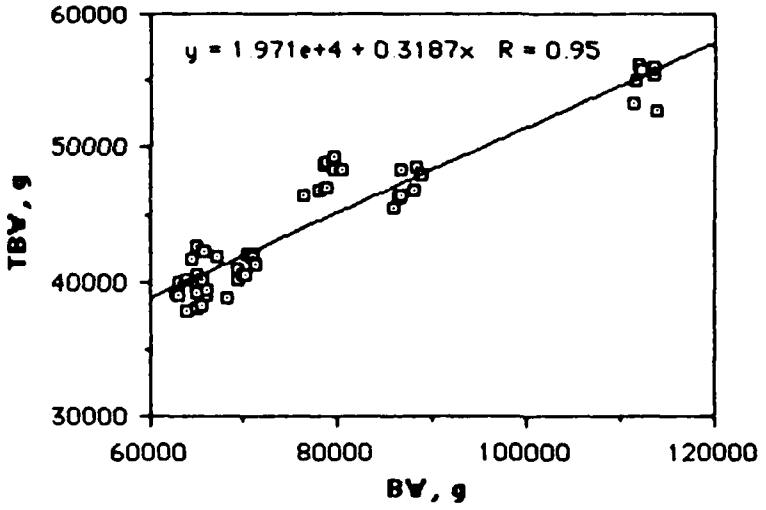

Fig. 11- Relationship between Total Body Water and Body Weight

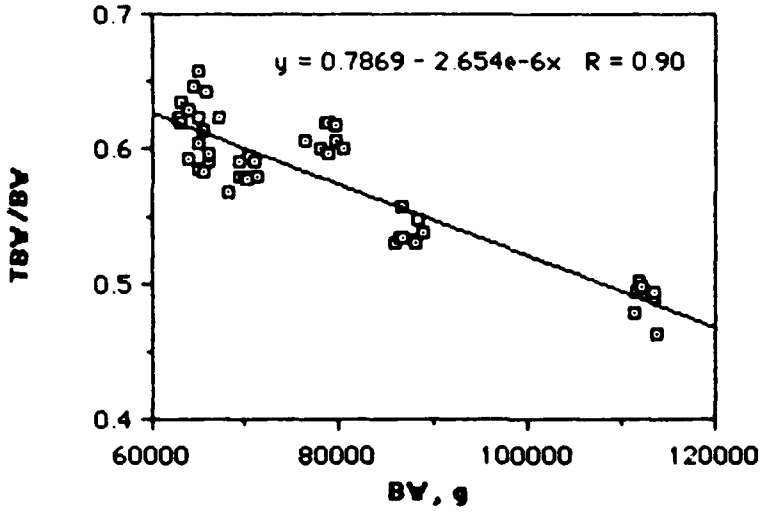

Fig. 12- Relationship between Total Body Water and Body Weight

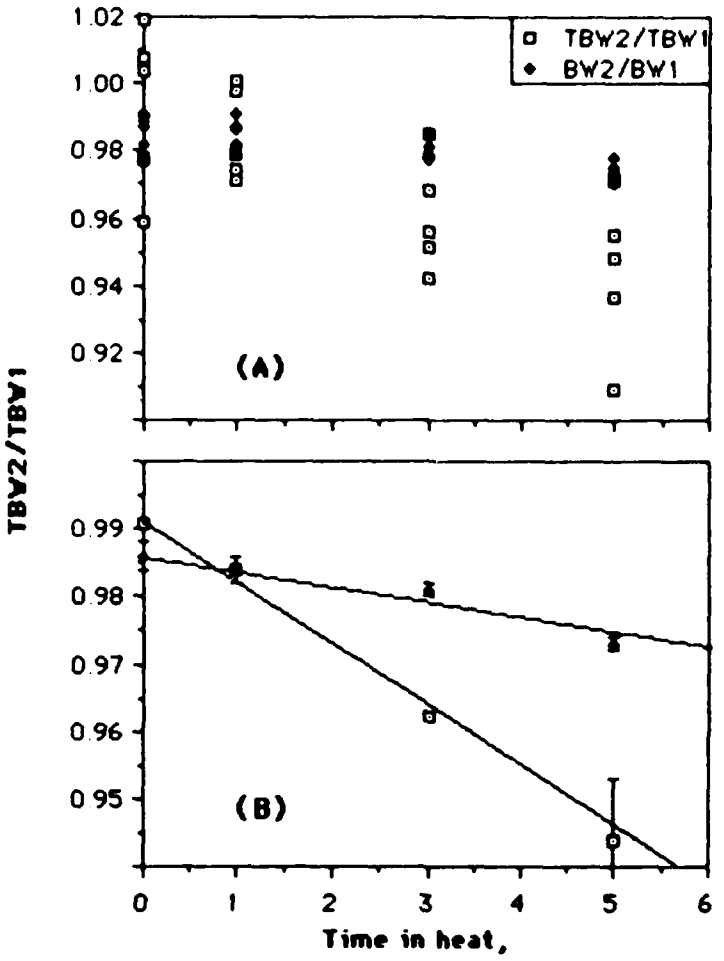

Fig. 13- Fractional Loss of Body Water (TBW) and Body Weight (BW) vs. Time in the Chamber (A) all data; (B) mean values±1SEM

1) Technical. A number of important improvements need to be made if this method is to achieve its full capability.

a) There are numerous potential sources of contamination of the samples with exogenous water. This must be controlled scrupulously.

b) Sufficient precision of isotope measurements is crucial to these studies. In the present experiment it was not possible to assure this by appropriate duplication of analyses due to the sheer size of the study (about 1200 samples were analyzed). Duplicate anlaysis is crucial to the satisfactory outcome of these studies. Therefore, future experiments should be designed with this important requirement in mind.

c) The dose of labeled water administered must be optimized in relation to the requirements of the study. In experiments requiring the ability to measure small changes in TBW, an appropriately higher dose of labeled water must be given. This can be calculated relatively precisely. In the present experiment, it was not possible to incease the dose level to assure maximum sensitivity. This was again due to the sheer size of the experiment and the limitations of the budget allowed for the isotope.

d) In experiments requiring sequential administration of multiple doses, appropriate procedures must be instituted to permit accurate measurement of drifting baseline ratios. This is a critical experimental parameter.

2) Fundamental Issues of Water Homeostasis.

A number of physiologically fundamental issues related to TBW and its homeostasis have emerged from this experiment. These need to be settled by careful experimentation before the method could be applied routinely:

a) What are the sources of the observed increase in TBW? Is delayed mixing with TBW more significant than realized heretofore?Does fasting contribute to this to an extent not appreciated previously? Or are our present observations consequent to some experimental artifact?

b) What are the fundamental relationships between quantitative loss of body water and body composition parameters? Is body size a critical parameter in relation to the ability to tolerate dehydration?

c) Is acclimatization to heat exposure a relatively rapid process? If so, can one design practical means of rapid acclimatization for personnel involved in rapid deployment?

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