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Optimum doses of deuterium oxide and sodium bromide for the determination of total body water and extracellular fluid

LARRY D. THOMAS,* DAVID VANDER VELDE† and PAUL R. SCHLOERB*‡

* *Department of Surgery, University of Kansas Medical Center, Kansas City, KS 66103, USA*
† *Nuclear Magnetic Resonance Laboratory, University of Kansas, Lawrence, KA 66045, USA*

Abstract: A practical approach for determining optimum tracer doses is described for measurements of total body water (TBW) and extracellular water (ECW) based on dilution of deuterium oxide and sodium bromide with respective analyses by nuclear magnetic resonance and anion-exchange chromatography. Using these techniques and plasma concentrations corresponding to adult doses up to 1.5 g kg^{-1} body weight of deuterium oxide and 0.05 g kg^{-1} of sodium bromide, the variations of analyses of these tracers, at these respective doses, were calculated. TBW determination with an RSD of less than 2% was found to require administration of 0.4 g kg^{-1} of deuterium oxide. Because basal concentrations of bromide are quantifiable, the accuracy of the extracellular water determination depends upon the magnitude of the increase in plasma bromide concentration; a sodium bromide dose of 0.01 g kg^{-1} provides a deviation in the determined ECW volume of approximately 1%.

Keywords: *Total body water; extracellular fluid; deuterium; bromide; nuclear magnetic resonance spectroscopy; anion-exchange chromatography.*

Introduction

Total body water (TBW) and extracellular water (ECW) determinations are useful for the evaluation of body composition and nutritional status. Water associates predominantly with non-fat tissue, thus TBW content serves as a basis for estimation of lean body mass. Simultaneous measurement of the two physiological compartments allows for the estimation of intracellular fluid volume and body cell mass [1]. In nutritional practice, use of lean body mass or body cell mass as a reference for nutritional support is preferable to use of body weight because of varying amounts of fat in men and women and variations with age [1].

Dilution of known amounts of tracer compounds is the most commonly utilized technique for measurement of TBW and ECW. A commonly used compound for TBW determinations is deuterium oxide (^2H_2O), which has about the same distribution volume as water [2] and is non-toxic at levels required for accurate quantitation. Deuterium exchanges with non-aqueous hydrogen that is 4–5% of that associated with water [2, 3]. For ECW volume, bromide is frequently utilized. Its well-characterized advantages include a distribution volume that closely represents ECW, rapid and complete absorption, slow clearance, and non-toxicity of tracer amounts [4].

For quantitation of these tracers in biological fluids, various analytical techniques have been employed. Methods for ^2H_2O include the falling drop method [5], gas chromatography [6], mass spectrometry [7, 8], infrared absorption [9], and deuterium nuclear magnetic resonance (^2H NMR) [10, 11]. The ^2H NMR method is increasingly used, because it is simple, fast, accurate, and does not require extensive sample preparation and utilizes equipment which is commonly available at universities and other research centres.

For the analysis of bromide, two liquid chromatographic methods are preferable, because of disadvantages and limitations associated with the other techniques. The method of Wong *et al.* [12] uses ion chromatography (IC), with UV detection at 210 nm and 1:10 dilution of plasma samples. That of Miller and Cappon [13] uses anion-exchange chromatography, UV detection at 190 nm, and no dilution. The anion-exchange method is sensitive and selective and, in this laboratory, has performed accurately and precisely for plasma samples.

‡ Author to whom correspondence should be addressed.

Although the NMR and anion-exchange methods are employed frequently, determinations of the optimum doses of tracers have not been conducted. Some authors, such as Wong [12], have discussed deviations in the volume determinations, but these are specific to their analytical techniques and generally examine only a single dose. In the present work, the variation of the TBW and ECW measurements with the dose of the respective tracers was studied to allow for the determination of optimum doses based on the acceptable levels of uncertainty in the determined volumes.

Experimental

Deuterium oxide analysis

Sixteen plasma samples were obtained from blood collected after administration of varied amounts of ^2H_2O as part of a clinical research project to measure body composition in patients with spinal cord injury. Additionally, 32 solutions were prepared by adding ^2H_2O (Sigma, St Louis, MO, USA) to deionized water, with subsequent volumetric dilutions. Deuterium oxide concentrations ranged from 2.5 mg ml^{-1} down to that of natural abundance.

The plasma samples and standard solutions were analysed by a modification of the NMR method of Khaled *et al.* [10]. Samples (0.3 ml) were placed in precision 5-mm NMR tubes (Norell, Inc., Mays Landing, NJ, USA). A sealed 2-mm coaxial insert containing a mixture of acetone and d_6-acetone (chosen to give approximately a 1:1 integral ratio of deuterated acetone and water when the ^2H_2O concentration is 1 mg ml^{-1}) was inserted in each tube. The ^2H NMR spectrum was then taken five times on a Bruker AM-500 spectrometer. Each spectrum consisted of four dummy scans and 128 normal scans, acquisition time 2 s, 45° pulse (chosen to avoid saturation of the acetone resonance, whose T_1 is approximately 4 s). The spectra were carefully phased and the baselines corrected using a non-linear 'spline' fit provided in the Bruker applications software. Each peak was integrated over a region ±50 Hz from its maximum, and the integral ratios and the relative standard deviations (RSD) calculated. Propagation of error calculations indicate that these RSDs closely approximate the RSDs of the determined TBW volume, since other sources of errors were negligible. After correcting the measured concentrations for the volume effect of solids in plasma (95%, v/v), corresponding adult doses were calculated assuming a distribution volume of 60% of body weight [2].

Bromide analysis

Portions of plasma, with added sodium bromide (J.T. Baker Chemical Co., Phillipsburg, NJ, USA) to give concentrations ranging from 0.0012 to 0.25 mg ml^{-1}, were deproteinated with Amicon Centrifree micropartition systems, 30,000 molecular weight cut-off (Amicon, Danvers, MA, USA). The ultrafiltrates were analysed by a modification of the anion-exchange method of Miller *et al.* [12].

The chromatographic determinations employed an integrated Shimadzu 6A system: SCL-6A system controller, LC-6A pump, SIL-6A autoinjector, SPD-6A spectrophotometric detector, and C-R3A data processor (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). The analytical column was a Partisil SAX (250 × 4.6 mm i.d., 10 μm particle size, Whatman, Inc., Clifton, NJ, USA), equipped with an anion-exchange guard column (20 × 2 mm i.d., 30–40 μm pellicular particles. Alltech Associates, Inc., Deerfield, IL, USA). The separation was carried out at ambient temperature, using a mobile phase of potassium dihydrogen phosphate (no pH adjustment, 0.015 M) at a flow rate of 0.7 ml min^{-1}. The KH_2PO_4 was purchased from Sigma Chemical Co. (St Louis, MO, USA) and reagent-grade water was obtained from a Milli-Q system (Millipore, Milford, MA, USA). The detection wavelength was 195 nm and the injection volume 20 μl.

For each sample, five injections were made, and the RSD of the measured peak areas was calculated. These values were used to calculate the uncertainty in the difference in peak areas of samples obtained before administration and after equilibration of the tracer. Assuming that the uncertainty of the slope of the calibration curve was negligible, this quantity was considered equivalent to the error of the increase in concentration of bromide in plasma, which was the only source of appreciable error in the determined ECW volume. Calculations modelled standard treatments for the propagation of errors [14, 15]. Tracer doses corresponding to the concentration of bromide in the samples were calculated, assuming a distribution volume of 20% of body weight [1].

Results

The TBW and ECW measurements in patients were found to be within the range of expected values. None of the clinical measurements was repeated. Figure 1 presents the relationship between tracer dose (x) and RSD for volume determinations of TBW (y). The best least squares fit of the data was:

$$y = 6.02e^{-6.38x} + 1.40e^{-0.0141x} \quad (1)$$

with a correlation coefficient (r) of 0.86. A TBW determination with an RSD of less than 2% required administration of 0.4 g kg^{-1} of deuterium oxide.

For sodium bromide samples, which ranged in concentration from 0.012 mg ml^{-1} (representing the basal concentration) to 0.25 mg ml^{-1}, RSDs of the measured peak areas were 0.71 and 0.17% for the respective lower and upper ends of the concentration range. The corresponding RSDs for the volume determinations of ECW (y) are presented, as related to tracer dose (x), in Fig. 2. The best fit of the data using least squares was:

$$y = 1.57e^{-38.72x} + 9.64e^{-1961x} \quad (2)$$

with a correlation coefficient (r) of 0.99. Therefore, a dose of 0.01 g kg^{-1} body weight provided an RSD in the determined ECW volume of about 1%.

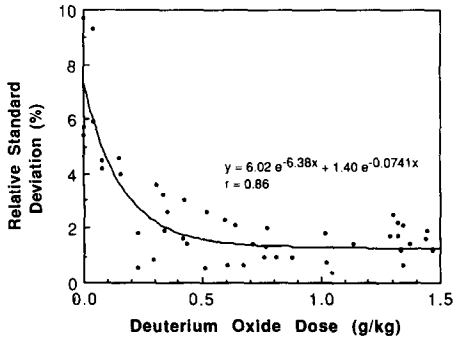

Figure 1
Plasma samples and water containing deuterium oxide were used to determine the RSDs for the measurement of total body water. "Dose" is the amount of tracer per kilogram body weight assuming a distribution volume of 60% of body weight.

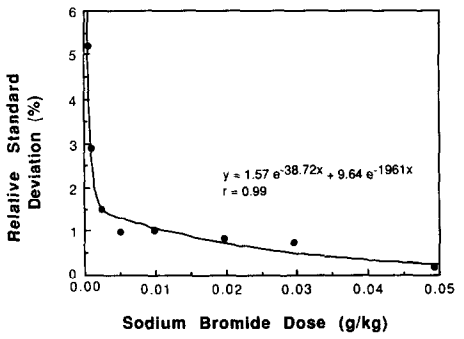

Figure 2
The RSDs for extracellular water volume, as determined using plasma containing sodium bromide. "Dose" assumes a distribution volume of 20% of body weight.

Discussion

While amounts of tracers administered should be large enough to allow for accurate and precise quantitation, they should be minimized for several reasons. Although deuterium oxide and sodium bromide are considered non-toxic in tracer amounts, reduced quantities should translate into reduced possibilities of physiological effects due to the tracers themselves. Repeated determinations in a single subject are facilitated by the use of small doses which help to prevent excessively high background concentrations. The results of the present study show that tracer doses may be determined using the plots of RSD versus dose (g/kg). The optimum doses are taken to be those corresponding to the acceptable variation in the volume measurements.

Comparison of the variation of our NMR measurements with those of others, indicated that the level of precision in the present study was intermediate. Khaled *et al.* [10] reported RSDs of 3 and 0.6% for concentrations corresponding to respective doses of 0.067 and 0.27 g ^2H_2O kg^{-1}. Calculations of RSDs for equivalent levels using the equation of this study yield values of 5.3 and 2.5%, respectively. Rebouche *et al.* [11] cite RSDs of 4.8 and 2.0% for levels of 0.40 and 1.3 g ^2H_2O kg^{-1}, respectively. From the present NMR work, these levels translate into respective RSDs of 1.8 and 1.3%. While the approximate agreement between laboratories gives credence to the data in this study, the difference points to the fact that particular instrumental and experimental conditions, e.g. probe size, influence the degree of precision, and thus the optimum tracer dose. Likewise, for the bromide analysis, conditions specific to the HPLC instrumentation used can alter the precision obtained.

Conclusions

For determination of total body water, deuterium oxide dilution with measurement of the tracer by NMR is the most readily available approach. Although greater precision in ^2H_2O analysis is afforded by the isotope ratio mass spectrometer, that of the NMR spectrometer, using larger amounts of deuterium oxide, is acceptable. For measurement of bromide, anion-exchange chromatography provides acceptable accuracy and precision.

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