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DETERMINATION OF BODY WATER CONTENT USING TRACE LEVELS OF DEUTERIUM OXIDE AND INFRARED SPECTROPHOTOMETRY

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FOREWORD

This report was prepared in the Pathology Branch under task No. 775511. The work was accomplished between December 1966 and September 1967. The paper was submitted for publication on 5 December 1967.

The deuterium oxide used in these studies was obtained from Nuclear Research Chemicals, Inc.

The authors are grateful to Marvin Lindsey for his assistance in the fabrication of the vacuum sublimation apparatus used in this study and to Master Sergeant James F. Green, Staff Sergeant Guy M. Strong, and Staff Sergeant John W. Harper of the Radiology Branch for their valuable assistance during the comparative studies involving tritium oxide dilution.

This report has been reviewed and is approved.

GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

A procedure is described for the determination of total body water content of human subjects by use of doses of 11 to 12 gm. of D_2O, vacuum sublimation of serum samples, and quantitation of the deuterium by infrared spectrophotometry at 2,510 $cm.^{-1}$ Experimental data are presented relevant to in vivo equilibration of D_2O, day-to-day reproducibility of sublimation and spectrophotometric assay, recovery of added D_2O, and results obtained with the procedure compared to a technic for tritium oxide dilution. The coefficient of variation based on day-to-day reproducibility of procedure is less than 2%. D_2O added to serum is essentially completely recovered in the sublimation and assay operations. The D_2O dilution procedure compares favorably with the tritium dilution technic. Values obtained on 45 out of 46 individuals fall within the $\pm$ 3 combined S.D. limits for the two methods.

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I. INTRODUCTION

Deuterium oxide dilution has been extensively used for the measurement of total body water content in mammals. A large volume of experimental evidence indicates that D_2O is readily absorbed by the gastrointestinal tract and equilibrates with the body water within a few hours. On the basis of a limited number of deuterium estimations in urine and plasma, there does not seem to be a significant degree of selective excretion of D_2O by way of the kidney. In addition, deuterated water is not radioactive and is nontoxic in tracer amounts (1). Numerous comparative investigations have indicated close agreement among the volumes of total body water determined through deuterium dilution and tritium dilution, and those measured directly by desiccation (2, 3).

Deuterium oxide in plasma has, in the past, been quantitated by a variety of methods including the falling drop technic (4), mass spectrometry (5), freezing point elevation (6), gas chromatography (7), and infrared absorption (8, 9). Invariably, however, the sensitivities of the methods have demanded the administration of relatively large doses of D_2O to subjects or have, in themselves, been laborious and time consuming.

This paper describes a method for determining total body water content in human subjects using deuterium oxide dilution, vacuum sublimation of plasma samples, and quantitation by means of infrared absorption (9, 10). The chief advantages of the procedure are the relatively small dose of D_2O (10 ml.) needed for adult subjects and the ease of assay as well as satisfactory accuracy and precision.

II. BASIS OF ASSAY

When pure deuterium oxide and pure water are mixed, the following equilibrium occurs:

$$H_2O + D_2O \rightleftharpoons 2\,HOD$$

The equilibrium constant for the reaction is

$$K = \frac{[HOD]^2}{[H_2O]\,[D_2O]} = 3.80 \text{ at } 25^\circ \text{ C.}$$

When the deuterium concentration is less than 3 vol. %, the solution is comprised essentially of only the two molecular species, H_2O and HOD. The concentration, then, of 0 to 1 vol. % of D_2O can be adequately assayed by measurements of the O-D fundamental vibration band of HOD at 3.98 μ (2,513 cm.$^{-1}$). Since this point is specifically associated with HOD, it is possible to make quantitative measurements of this structure at 2,513 cm.$^{-1}$ when the HOD is in an aqueous distillate from a more complex system.

III. MATERIALS AND METHODS

Reagents

The deuterium oxide used in this study was 99.76% (w/w) enriched. The purity of the D_2O solution was verified by measuring its density at 20.00° ± 0.01° C. and by the near-infrared

spectrophotometric procedure of Keder and Kalkwarf (11). When deionized, degassed water was used for reference and dilution purposes, it was assumed to contain the natural background D_2O concentration of 0.014 vol. %.

Serum used as a matrix for preparing daily control samples was prepared from a pool of normal human serum which had been filtered through a Seitz filter to remove gross particulate material. This filtered serum was also used in the recovery studies.

Concentrations of D_2O cited in this study are D_2O added to the existing diluent materials. Deuterium oxide aqueous calibration standards were prepared gravimetrically using a Mettler semi-micro balance. All purely aqueous solutions were stored at 4° C. in tightly sealed containers in which there was essentially zero dead airspace. This was necessary to preclude gradual exchange of D_2O with atmospheric H_2O during periods of storage. Control specimens prepared in serum matrices were subdivided into 5-ml. aliquots and quickly frozen. On each working day a fresh set of aliquots covering the full concentration range was thawed and processed.

Vacuum sublimation of serum samples

Vacuum sublimation of H_2O-D_2O solutions from serum samples was performed in the apparatus depicted in figure 1. The cooling bath was a system of solid CO_2 and isopropanol. Vacuum was supplied by a Welch Scientific R1405 vacuum pump, and pressures in the order of 0.05 mm. Hg were routinely employed. All interconnections were kept as short as possible to minimize diffusion distances.

Sample and reference cells

An uncalibrated Beckman sealed liquid cell with calcium fluoride windows and a fixed 0.1-mm. spacer was employed as the reference cell in this study. A similar cell with a fixed 0.2-mm. spacer was used for all samples. A Beckman reference beam attenuator was necessary in the reference beam in order to achieve initial energy balance. The sample cell was provided with short extensions of Tygon capillary tubing which were secured to the frame of the spectrophotometer. These extensions, fitted with Luer-Lok connectors and a 3-way stainless steel surgical valve, permitted flushing and loading of the sample cell without having to remove it from its holder, thus eliminating a major source of analytic variance. A sintered glass filter was mounted in the cell inlet line to preclude entry of any stray debris.

Instrument

A Beckman IR-9 infrared spectrophotometer was used to measure absorbances. The instrument was situated in an atmosphere with relative humidity of approximately 60% and an ambient temperature of 22° to 24° C. No purging of the apparatus with CO_2-free dry nitrogen or air was found to be necessary (10) during routine operation.

Temperature control

Control of cell temperatures was achieved by circulating water of stable temperature through water jackets (10) into which the analytic cells were inserted. Water temperature was controlled by a Forma constant-temperature circulating water bath.

FIGURE 1

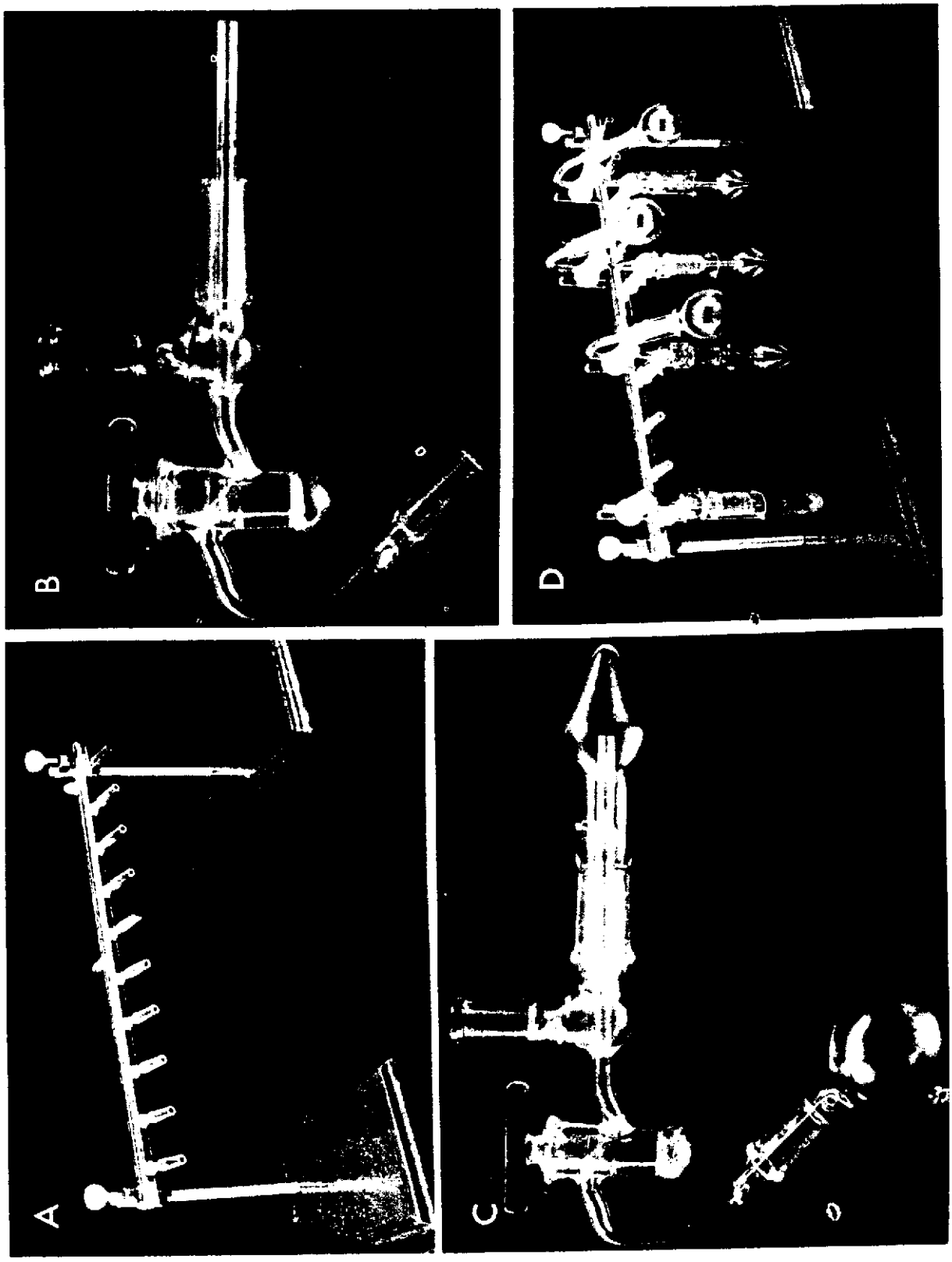
Vacuum sublimation apparatus.

A: *Manifold to which vacuum line and sublimation units are attached.*

B: *Basic sublimation unit with sample sublimation flask and receiving vessel removed.*

C: *Assembled sublimation unit. Samples are shell-frozen in and sublimated from the round bottom flask. The H_2O-HOD sublimate is collected in the conical-shaped receiving vessel.*

D: *Partially assembled vacuum sublimation apparatus. The unit on the far left serves as a bleeding valve. The conical-shaped receiving vessels are immersed in solid CO_2-isopropanol to just below the ground-glass joint.*


B
D
A
C

Routine adjustment of spectrophotometer

During routine operation, the sequence of activities was as follows:

1. The spectrophotometer glower and chopper circuits were energized at least 1 hour before beginning analyses. At this time, the reference cell containing deionized diluent water was placed in the reference beam and allowed to thermally equilibrate during the entire warmup period.

2. The gain, the period, and the slits controls were set to 1.0, 2, and manual, respectively. During intervals in which the sample cell was being flushed, dried with air, or otherwise being disturbed, the gain was always set to zero in order to minimize pen response.

3. With both filled reference and filled sample cells in place, the ratio of single beam to double beam was adjusted to 1:1 at 2,510 $cm.^{-1}$ The precautions discussed earlier (9, 10) were observed.

4. Immediately before recording, the instrument was set to operate from 90% to 100% transmittance (T).

5. All tracings were prepared at the single fixed wavelength of 2,510 $cm.^{-1}$ The recording paper was advanced beneath the pen for a period of approximately 1 minute. The resultant tracing reflected the background variation characteristic of high scale expansion. The relative position of the tracing on the scale was a function of the amount of D_2O in

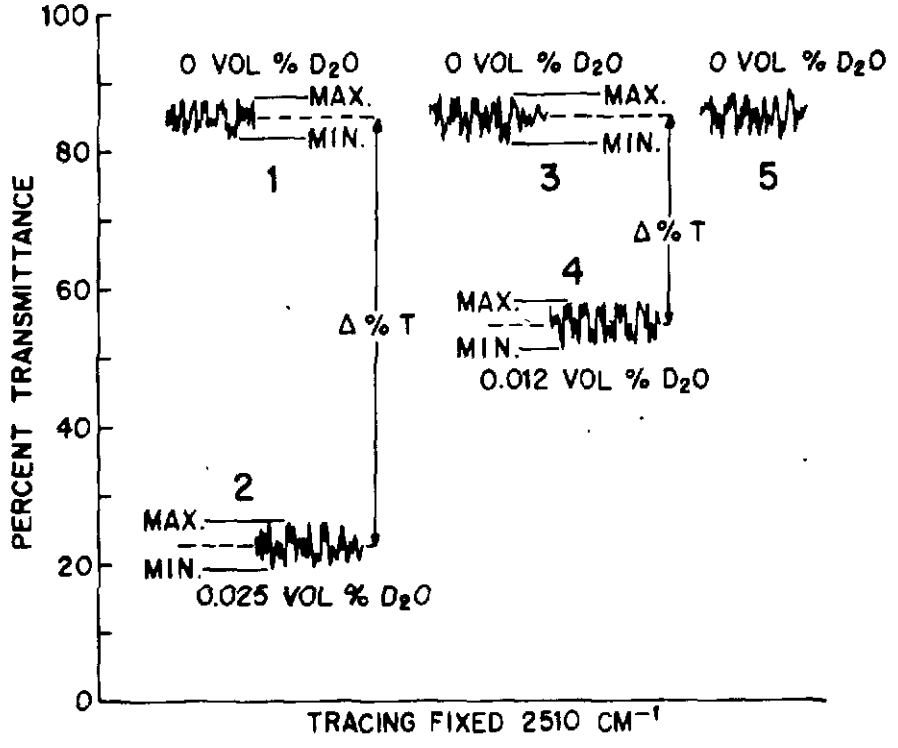


FIGURE 2

Typical tracings obtained for aqueous samples containing trace levels of deuterium. The Δ % T values employed in calculating deuterium concentrations are obtained by determining the percent transmittance increment between the midpoints of the tracing for 0 vol. % added D_2O and the tracing for the sample with added deuterium. The large Arabic numerals indicate the sequence of recording during normal operation.

the sample. All series of assays were commenced with the tracing, at approximately 85% T, of the absorbance pattern for the appropriate diluent with no added D_2O. This tracing was recorded as the baseline value, and subsequent measurements in that particular series of analyses were related to it. To minimize drift error, the sample with no D_2O was measured between all samples containing added deuterium. Figure 2 illustrates the application of the method.

The routine analysis of D_2O in serum sublimates was conducted with rigid control of the temperatures of the reference and sample cells. The temperatures of the reference and sample cell water jackets were equilibrated with those of external circulating water baths maintained at 48° and 30° C., respectively.

The filled reference cell (containing deionized diluent water) remained undisturbed in its water jacket throughout the series of analyses, and only the sample cell contents were varied.

At the beginning of each series of assays, the gain control was set to zero in order to prevent violent pen movement during cell charging. A 1-ml. syringe was filled with baseline sample (0 vol. % added D_2O) and then attached to the Luer connector coupled to the Tygon tubing. The sample was injected until it emerged from the outlet port of the cell. Approximately 0.4 ml. was required (0.1 ml. for flushing purposes and 0.3 ml. to load the cell). The syringe was left in place, and the gain control was advanced to 1.0. After adjusting the single beam – double beam ratio to 1:1, the recording paper was advanced beneath the pen.

After one minute of recording, the paper was stopped, and the gain control was returned to zero. The sample was then withdrawn from the cell into the attached syringe, and the syringe was removed. A stream of filtered, dry, compressed air was then used to completely dry the interior of the cell before introducing the next sample. This procedure was duplicated as nearly as possible for each additional sample.

Determination of total body water

Adult male subjects were instructed to abstain from food and water after 2200 hours the night before the scheduled determination. On the morning of the determination at approximately 0730 hours an 8- to 10-ml. sample of blood was withdrawn and immediately capped and set aside to clot. An accurately weighed sample of D_2O was quantitatively given orally before approximately 25 ml. of deionized diluent water were given. The weight of D_2O administered ranged between 10.9628 and 12.1775 gm. The subject was instructed to remain in a quiet state during the equilibration period. Midway through the equilibration period the subjects were weighed to the nearest 0.01 kg. Approximately 3 hours after D_2O dosage, a second sample of blood was collected, capped, and permitted to clot.

The capped samples were centrifuged, and 1.7 ml. of the serum were transferred to 50-ml. sublimation flasks. The serums were shell-frozen using a slurry of Dry Ice and isopropanol and then vacuum-sublimated. From a 1.7-ml. serum sample, 1.5 ml. of H_2O-HOD solution could be obtained routinely in 60 minutes.

The pre-D_2O sample was used to obtain the baseline tracing. The percent T increment occurring between it and the tracing obtained using the post-D_2O equilibration sample was recorded. The concentration of D_2O in the solution was obtained using a standard curve prepared on the day of assay. The total body water content was then calculated from the assay value (concentration of postequilibration sample minus concentration of predose sample), the volume of D_2O administered, and the weight of the subject.

Calculations

By recording the temperature (t), the weight of 99.76% D_2O administered (W) can be converted into the volume of pure D_2O administered (V):

$$V\ (ml.) = \frac{W\ (gm.) \times 0.9976}{\text{Density of } D_2O \text{ at } t^\circ C.}$$

The total body water in liters

$$= \frac{\text{Volume } D_2O \text{ administered} - \text{Volume } D_2O \text{ excreted}}{10 \text{ (concentration of } D_2O \text{ in serum water in vol. \%)}}$$

The volume of D_2O excreted includes both insensible loss and urinary excretion. On the basis of the findings of Schloerb et al. (12), 0.5% of the administered D_2O volume was assumed to be excreted during the 3-hour equilibration period.

IV. RESULTS

Equilibration time

In order to confirm the validity of using 3 hours for in vivo D_2O equilibration, a series of total body water determinations was conducted on a group of 20 normal individuals. Samples of blood were obtained at ½, 1, 2, 3, and 4 hours after administration of D_2O. In all subjects equilibration was reached by 3 hours, and in 80% of them the D_2O level had reached a plateau by 2 hours.

None of the subjects tested exhibited grossly abnormal fluid accumulation. The turnover rate of deuterated water, which does not differ significantly from that of pure water, in large volumes of transudate (edema, ascites) is slow compared to the transfer and mixing rates within the remainder of the body. In cases of abnormal fluid accumulation, therefore, longer equilibration times (6 to 24 hours) would be required (13).

Reproducibility of standard curve

The day-to-day reproducibility of the standard curve is summarized in figure 3 and table I. The percentage variability (coefficient of variation) is substantially improved over that reported earlier (10) using a different technic. For example, at the level of 0.0157 vol. % (174 p.p.m. w/w) in the earlier study (10), the coefficient of variation is $\frac{0.67}{19.0} \times 100$, or 3.53%. The coefficient of variation of 1.41% at 0.0150 vol. % in the present study (table I), however, is less than half this figure. This improvement is due to the nearly double net percent T span obtained with the 0.2-mm. path length.

Day-to-day reproducibility of assaying sublimated samples

Three control serums were gravimetrically prepared to contain 0.0161, 0.0195, and 0.0231 vol. % added D_2O. These were actual aqueous compartment concentrations calculated from a measured 9.42% (w/w) solids content. Each of the serums was sublimated and assayed over a period of 10 days. The data of table II compared to those of table I indicate that the additional manipulation attendant to the lyophilization procedure apparently does contribute to the inherent variability of the assay. The mean assay value in each case, however, is in satisfactory agreement with the calculated value, thus suggesting that D_2O is not being

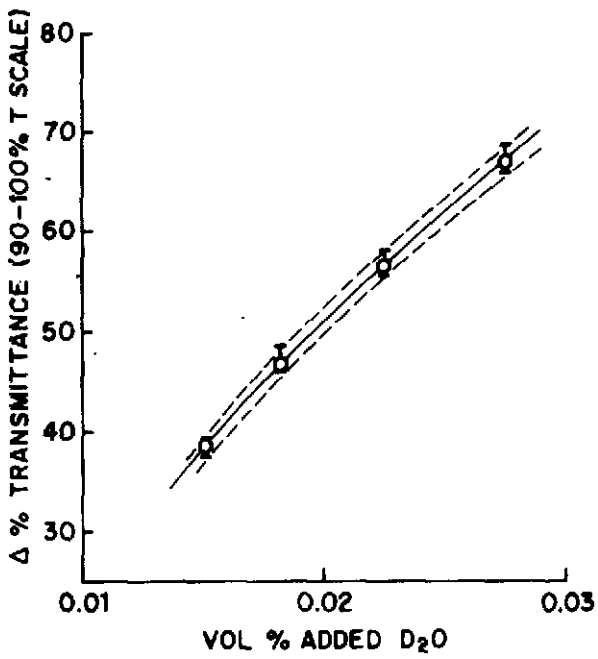


FIGURE 3

Day-to-day reproducibility of the standard curve. The vertical bars indicate the Δ % T range obtained for each concentration over a period of 19 days. The solid line and circles represent the mean values. The broken lines on either side of the mean limit ± 2 S.D.

TABLE I

Reproducibility of standard curve

Concentration of standard* (vol. %)	Number of days	Range (Δ % T)	Mean (Δ % T)	S.D. (% T)	C.V. (%)
0.0150	19	37.5–39.0	38.3	.54	1.41
0.0182	19	46.0–48.5	46.8	.64	1.37
0.0224	19	55.5–58.0	56.5	.69	1.22
0.0274	19	66.0–68.5	67.2	.71	1.05

*All standards used in these studies were prepared as pure H_2O-HOD solutions and stored in the fluid state in closed containers. They were routinely measured directly in the spectrophotometer without passing through the sublimation procedure.

TABLE II

Day-to-day reproducibility of assaying sublimated samples

Prepared concentration* (vol. %)	Number of days	Spectrophotometric readings			Assay				
		Range (Δ % T)	Mean (Δ % T)	S.D. (% T)	Range (vol. %)	Mean (vol. %)	S.D. (vol. %)	C.V.† (%)	Error† (%)
0.0161	10	39.2–41.0	40.3	.69	0.0157–0.0162	0.0159	.00027	1.7	−1.24
0.0195	10	47.0–49.0	48.0	.78	0.0189–0.0198	0.0193	.00034	1.7	−1.02
0.0231	10	55.5–57.6	56.6	.89	0.0225–0.0233	0.0230	.00038	1.6	−0.44

*Gravimetrically prepared.

†Percent error $= \frac{\text{Mean assay concentration (vol. \%)} - \text{Prepared concentration (vol. \%)}}{\text{Prepared concentration (vol. \%)}} \times 100.$

lost during the process. During the early stages of experimentation, the samples were not routinely sublimated to near total dryness, and the lyophilization step failed to consistently yield the calculated HOD concentration. It appears that preferential distillation of H_2O (D_2O has 8.8% lower vapor pressure than H_2O at 20° C. (14)) contributes to decreased D_2O recovery if the sample is not taken to dryness. The small amount of tenaciously bound water remaining in the residue apparently does not contribute significantly to low yields of D_2O in the sublimate.

Recovery studies

Increments of pure D_2O were gravimetrically added to a series of stable control serums so that the resultant D_2O concentrations would resemble those encountered in performing body water determinations. The samples were then carried through the sublimation procedure, and the sublimates assayed spectrophotometrically. These recovery studies were conducted over a period of 3 days. The results (table III) indicate acceptable recovery of the expected HOD concentrations. The influence of day-to-day variability in the total procedure is evident from the recovery levels tabulated for the three different days. On day 1 the recovery levels all tend to be slightly less than 100%, with one value (94.6%) reflecting the occasional deviant measurement encountered with any procedure. On day 2 the values tend to be in excess of 100%, and the results of the third day are similar to those of day 1.

Comparison with reference method

The tritium dilution procedure of Prentice et al. (15) has for several years been the standard method employed at the USAFSAM for estimating body water content. Reproducibility studies conducted with this procedure indicate a standard deviation of 0.8 liter at the 38-liter level. This value was employed in all inter-method comparisons.

A group of 46 male subjects was tested using both the reference T_2O dilution method and the D_2O procedure. Figure 4 summarizes the data in terms of liters of body water. By use of the scheme proposed by Barnett (16), the mean of the assay values obtained with the deuterium dilution method was found to be 0.3 liter lower than that obtained with the tritium dilution technic. The Student's t-test revealed that the difference between the two procedures is not statistically significant. A combined standard deviation was calculated using the standard deviation of 0.8 liter for the T_2O method and 0.71 and 0.90 liters at the 43- and 50-liter levels, respectively, for the D_2O procedure. These values were interpolated from table II, assuming a standard volume of 10 ml. of pure D_2O being administered to each subject. From figure 4 it is evident that one value is excluded by the ± 3 combined S.D. limits for the two methods. In a total number of 46, one such deviant value is not sufficient to disqualify the test method.

TABLE III

Recovery of D_2O added to serum matrix

Sample No.	D_2O added (vol. %)	D_2O assayed* (vol. %)	Recovery (%)
Day 1			
1	0.0150	0.0148	98.7
2	0.0159	0.0158	99.3
3	0.0163	0.0154	94.6
4	0.0167	0.0163	97.8
5	0.0176	0.0174	99.0
6	0.0180	0.0178	99.0
Day 2			
7	0.0195	0.0200	102.5
8	0.0198	0.0201	101.5
9	0.0201	0.0204	101.5
10	0.0203	0.0203	100.0
11	0.0206	0.0207	100.5
12	0.0209	0.0215	102.9
13	0.0211	0.0216	102.2
14	0.0214	0.0217	101.2
15	0.0225	0.0228	101.1
Day 3			
16	0.0236	0.0232	98.4
17	0.0261	0.0259	99.3
18	0.0264	0.0256	97.1
19	0.0326	0.0319	98.0
		Av.	99.8

*Average of triplicate spectrophotometric assays.

The data of figure 5 present the liter values as percent body water. These data were obtained by dividing the liter value determined for each subject by the total body weight of the subject in kilograms. The mean of the values calculated from the deuterium dilution data is 0.9% lower than that computed from the tritium dilution results. The combined S.D. was calculated for the points indicated. A 75-kg. body weight was assumed in each case. The ± 3 combined S.D. limits for the two methods barely exclude one value, suggesting that all values obtained are within the combined inherent variabilities of the test and reference procedures. The increased variability observed when the volume data are presented as percent body water values is due to the inclusion of an additional parameter, body weight. Two volume values, therefore, of 40.0 and 40.5 liters (differing by 0.5 liter) could result in values of 66.7% and 67.6% for percent body water at body weight 60 kg. or 40.0% and 40.5% at 100-kg. body weight.

It is common practice to use the values for body water (liters) to calculate percent lean body mass (L.B.M.) and percent body fat (B.F.). The lean body mass is considered to be equal to the whole body minus nonessential or excess lipids. Essential lipid is estimated to be 2% of the lean body weight.

Since water constitutes, on the average, 73% of the fraction remaining after subtracting fat, percent body water = 0.73 (100 — percent fat) (15). Using the relationships

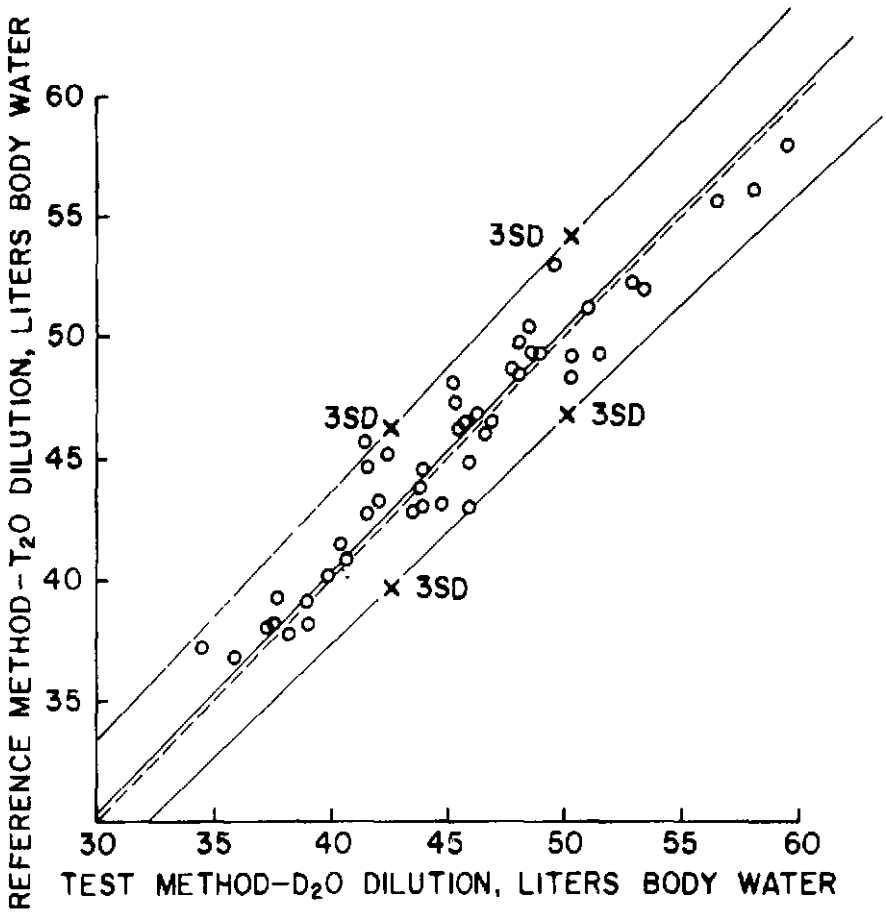


FIGURE 4

Comparison of T_2O dilution procedure and deuterium dilution method with respect to values for liters of body water. The significance of the plotted data is discussed in the text. The broken line emanates from the origin and bisects the plot. The solid line is parallel to the broken one (16) and reflects the fact that the mean of the values obtained with the D_2O procedure is 0.3 liter lower than that obtained with the T_2O dilution method.

$$\text{L.B.M.} = \frac{\text{Percent body water}}{0.73}$$

and

$$\text{B.F.} = 100 - \text{L.B.M.}$$

the average L.B.M. for the test group of 46 subjects was calculated to be 76.1% and the average B.F. was computed to be 23.9%.

V. DISCUSSION

A small error is probably introduced owing to an exchange of deuterium with labile hydrogen atoms of serum organic molecules, primarily proteins. Because of this rapidly occurring exchange, ingested and absorbed deuterium will be diluted into a volume slightly greater than that occupied by the body water alone. This error of overestimation has been calculated by Schloerb et al. (12) to be 0.5% to 2.0% of the body weight. A correction for these exchanges is normally not applied to measurement of body water using D_2O where the objective is to observe changes in body water within an individual. In this instance, the error due to exchange is unimportant since it will remain relatively constant. For absolute measure-

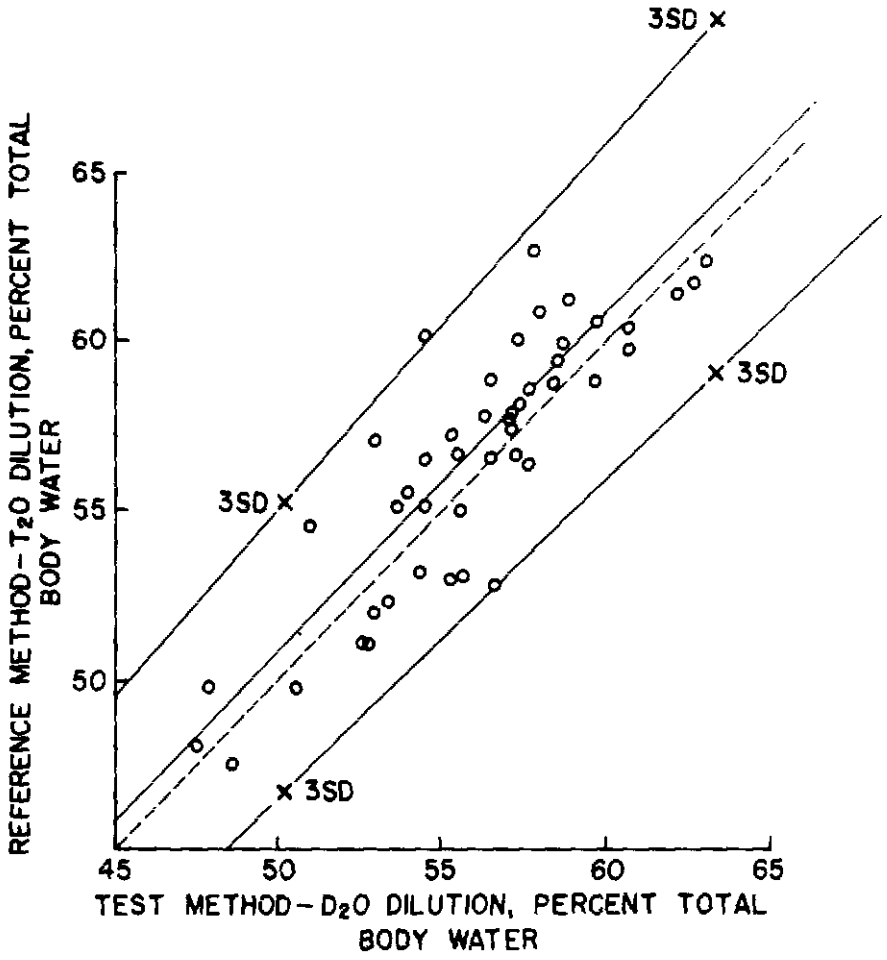


FIGURE 5

Comparison of T_2O dilution procedure and deuterium dilution method with respect to values for percent body water. The significance of the plotted data is discussed in the text. The broken line emanates from the origin and bisects the plot. The solid line is parallel to the broken one (16) and reflects the fact that the mean of the values obtained with the D_2O procedure is 0.9% lower than that obtained with the T_2O dilution method.

ments of water, however, a correction of about 1% of the body weight is needed to more closely approach the actual value.

The recent report of Graystone et al. (17) indicates that values obtained for body water using D_2O dilution and infrared spectrophotometry are approximately 20% higher than values obtained using D_2O and the falling drop technic. Since their study involved quantitation of D_2O in a vacuum distillate by four different technics and infrared spectrometry yielded the lowest recovery of the four approaches, it can be inferred that the low recovery of D_2O using IR was due to a substance in the distillate which interfered with the 2,510 cm.$^{-1}$ absorbance or that the IR technic itself was faulty. The recovery data from the present method support the absence of any interfering materials and the adequacy of the analytic technic. In addition, the comparison between deuterium dilution and tritium dilution discussed above shows that the D_2O-IR procedure yields body water values tending to be slightly lower than those obtained with a method using an entirely different hydrogen isotope.

Even if values for the body water are determined with great accuracy, they must be interpreted cautiously in relation to fat content. Siri (13) estimated that the range of hydration may normally vary ± 6.5% of the total body water. From an interpretive point of view this means that a body water content can vary ± 6.5% from the individual's mean value and still not be attributed to differences in body fat.

In the leanest possible body (one devoid of nonessential lipid) the total body water is approximately 72% of the body weight (13). Total body water content has been seen as low as 38% of the body weight (13). The average value found for body water content in the test group is nearly 7% lower than the 62% (v/w) average body water content usually reported for adult males (18). In the subpopulation sample studied, only 3 individuals (6.5%) exhibited body water contents equal to or greater than the normally accepted value of 62%. The possible significance of this observation in relation to subject dehydration or generally high storage fat level is currently being investigated.

REFERENCES

1. Pinson, E. A. Water exchanges and barriers as studied by the use of hydrogen isotopes. Physiol. Rev. 32:123-134 (1952).

2. Moore, F. D. Determination of total body water and solids with isotopes. Science 104:157-160 (1946).

3. Pace, N., L. Kline, H. K. Schachman, and M. Harfenist. Studies on body composition. IV. Use of radioactive hydrogen for measurement in vivo of total body water. J. Biol. Chem. 168:459-469 (1947).

4. Hytten, F. E., N. Taggart, W. Z. Billenwicz, and A. C. Jason. Estimations of small concentrations of deuterium oxide in water by the falling-drop method. Phys. Med. Biol. 6:415-425 (1962).

5. Friedman, I. Analysis of deuterium oxide by mass spectrometry. Geochim. Cosmochim. Acta 4:89-98 (1953).

6. Reaser, P. B., and G. E. Burch. Determination of deuterium oxide in water by measurement of freezing point. Science 128:415-417 (1958).

7. Arnett, E. M., and P. McDuggleby. A rapid and simple method of deuterium determination. Anal. Chem. 35:1420-1424 (1963).

8. Turner, M. D., W. A. Neely, R. Adcock, and A. C. Turner. A micromethod for the determination of deuterium oxide in biological fluids. Microchem. J. 8:395-401 (1964).

9. Stansell, M. J., L. Mojica, Jr., and H. H. Malvin. Measurement of trace levels of deuterium oxide in biologic fluids using infrared spectrophotometry. SAM-TR-67-37, Apr. 1967.

10. Stansell, M. J., L. Mojica, Jr., and H. H. Malvin. Quantitation of trace levels of deuterium oxide in water using infrared spectrophotometry. SAM-TR-67-13, Feb. 1967.

11. Keder, W. E., and D. R. Kalkwarf. Near-infrared determination of deuterium oxide in water. Anal. Chem. 38:1288-1291 (1966).

12. Schloerb, P. R., B. J. Friis-Hansen, I. S. Edelman, A. K. Solomon, and F. D. Moore. J. Clin. Invest. 29:1296-1310 (1950).

13. Siri, W. E. The gross composition of the body. *In* Lawrence, J. H., and C. A. Tobias (eds.). Advances in biological and medical physics, vol. 4, pp. 239-280. New York: Academic Press, 1956.

14. Siri, W. E. Isotopic tracers and nuclear radiations, pp. 263-267. New York: McGraw-Hill Book Co., 1949.

15. Prentice, T. C., W. E. Siri, N. I. Berlin, G. M. Hyde, R. J. Parsons, E. E. Joiner, and J. H. Lawrence. Studies of total body water with tritium. J. Clin. Invest. 31:412-418 (1952).

16. Barnett, R. N. A scheme for the comparison of quantitative methods. Amer. J. Clin. Path. 43:562-569 (1965).

17. Graystone, J., J. Seitchik, R. Milch, G. P. Shulman, and D. B. Cheek. Measurement of D_2O in plasma water by freezing point elevation, falling drop, infra-red absorption and gas chromatography. J. Lab. Clin. Med. 69:885-892 (1967).

18. Chien, S., and M. I. Gregersen. Determination of body fluid volumes. *In* Nastuk, W. L. (ed.). Physical techniques in biological research, vol. 4, pp. 1-105. New York: Academic Press, 1962.

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13 ABSTRACT

A procedure is described for the determination of total body water content of human subjects by use of doses of 11 to 12 gm. of D_2O, vacuum sublimation of serum samples, and quantitation of the deuterium by infrared spectrophotometry at 2,510 $cm.^{-1}$ Experimental data are presented relevant to in vivo equilibration of D_2O, day-to-day reproducibility of sublimation and spectrophotometric assay, recovery of added D_2O, and results obtained with the procedure compared to a technic for tritium oxide dilution. The coefficient of variation based on day-to-day reproducibility of procedure is less than 2%. D_2O added to serum is essentially completely recovered in the sublimation and assay operations. The D_2O dilution procedure compares favorably with the tritium dilution technic. Values obtained on 45 out of 46 individuals fall within the ±3 combined S.D. limits for the two methods.

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