Total body water and the exchangeable hydrogen II. A review of comparative data from animals based on isotope dilution and desiccation, with a report of new data from the rat

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There are only a few reports in the literature in which total body water (TBW) has been measured by isotope dilution and checked by carcass desiccation in the same animal (14, 23, 35). From the studies of heavy water kinetics in animals and man (31) and from a previous experiment comparing direct and indirect methods in the rabbit (23), it would have seemed that the method of isotope dilution was biologically valid. However, a recent report (35) has suggested that the difference between the two methods was statistically significant in the rat and that the dilution method overestimated TBW by 12% of the desiccated rat value. It is the purpose of this paper to report an attempt to corroborate this finding.

MATERIALS AND METHODS

Twenty-one Charles River outbred albino rats were provided with only water for 24 h prior to the experiment. Before starting the experiment all syringes, tubes, and tin foil were weighed in a Mettler H51 scale (d = 0.01 mg) and the weight recorded. The rats were weighed twice during the 2-h period of tritiated water (THO) dilution: once immediately after the THO injection, and again prior to the heart’s blood sampling. The rats were weighed in a torsion balance placed on the preweighed tin foil, together with the tube that would be used for blood desiccation.

The injection of THO1 was made intraperitoneally with the rat under ether anesthesia. The average amount of THO injected was 1.02474 ± 0.004 ml. The injection was made with a 1 ml disposable plastic tuberculin syringe with a 27-gauge ½ needle. The injectate was made up as 0.85% NaCl prior to radiation standardization.

Two hours later the rats were killed with ether and, immediately, a thoracoabdominal incision was made. A sample of blood was obtained by direct heart puncture. This sample was collected in two tubes. One was used for tritium counting, the other to measure the dry weight of blood. The tube intended for determination of

1 The tritiated water was obtained from Cambridge Nuclear Co. It was used at a specific activity of 10 μCi/ml.
water in blood by desiccation was weighed immediately after sampling and again daily until constant weight. The tube in which tritium activity would be measured was weighed immediately after sampling and then sealed to prevent evaporation. The syringe used to sample blood from heart was also weighed before use and at the end to measure any blood retained in its walls.

The rat was skinned completely and eviscerated and all the viscera were cut into small pieces. The rat body and skin was then cut into less than 1 cm² pieces from the tail to the head, taking special care to break absolutely every major bone, and the skull. The rat was then placed in an oven at a constant temperature of 90°C. In the following days the rat with tin foil and tube was weighed until a constant weight was achieved. Once all the data were recorded the dry weight of the rat was calculated by adding the dry weight of the blood sample and the blood left in sampling syringe to the dry rat weight. Sixteen of the twenty-one rats passed some urine during the 2-h period preceding death. This urine and the blood left in sampling syringe to the dry rat weight was also measured by weight differences and analyzed weight loss during the period of 2 h between THO injection and sampling 4 (Table 1). The isotope-dilution methods for body composition have provided a series of reproducible methods for measurement of the total exchangeable sodium, total exchangeable potassium, and total body water. Measurements of the red cell volume, the plasma volume, and the extracellular phase are based on the same principle. A full account of these methods including their validation by cadaver analysis is described in other reports (10, 23, 25, 28).

### RESULTS

The average rat weight after THO injection was 230.26 ± 42.14 g, and the second weight, recorded 2 h later, i.e., prior to killing the rat, was 227.11 ± 83.7 g. Only 16 of 21 rats passed urine during this period. The average amount of urine voided during this period was 0.92 ± 0.2 ml. The insensible weight loss calculated for this 2-h period (body weight loss minus voided urine) was 2.23 ± 0.6 g which represents 0.99% of total body weight.

The average number of days required for desiccation to constant weight was 4.24 ± 0.35. During the first 24 h the average weight was reduced to 36.54% ± 7.3% of the initial weight. After 48 h of desiccation, the weight was further reduced to 30.26% ± 5.5%. During the following days the desiccation curve formed a plateau with very slight further reduction to a final volume of 29.80% ± 1.4% giving therefore a TBW measurement by desiccation of 70.20% ± 1.5%.

TBW calculated by TI0 dilution showed a figure of 71.38% ± 2.44% of body weight after correction for 0.99% weight loss during the period of 2 h between THO injection and sampling 4 (Table 1). Analysis of variance of TBW versus body weight showed a highly significant relation, both with the desiccation values (P < 0.0005, r = -0.78) (Fig. 1) and the dilution values (P < 0.03, r = -0.50) (Fig. 2). Covariance analysis of desiccation versus dilution showed no difference in slope (P > 0.9). There was a significance in variance (P < 0.001) and in means (P < 0.05) (Fig. 3).

### DISCUSSION

The isotope-dilution methods for body composition have provided a series of reproducible methods for measurement of the total exchangeable sodium, total exchangeable potassium, and total body water. Measurements of the red cell volume, the plasma volume, and the extracellular phase are based on the same principle. A full account of these methods including their validation by cadaver analysis is described in other reports from these and other laboratories (10, 23, 25, 28).

The initial phase of tritium distribution consists of rapid equilibration within the extracellular space (6). Arterial concentration reaches a maximum of 3–5 times the eventual equilibrium concentration within a few seconds. During the following 2 h the isotope must traverse the capillary membrane, mix with extracellular water, cross the cell membrane to mix with intracellular water, and reach equilibrium with water stores such as cerebrospinal fluid, synovial membranes, intestinal contents, and glandular acini and ducts (10, 31).
Derived Values

Values derived from total body water show a number of interesting correlations suggesting the validity of the method. Of these, the value for average intracellular potassium concentration is possibly the most significant. This is based on three completely separate isotope-dilution methods (tritium, potassium, and bromide). The total intracellular potassium and total intracellular water make a linear series with body weight and with total body water. The figure of 147 meq/l for intracellular potassium concentration is constant throughout a wide range of body weight and closely correlates with data from direct tissue analysis. This subject is described in detail elsewhere (27).

The studies with the very late equilibrium of these isotopes have been carried out (11) demonstrating that there are no serial anomalies after the initial equilibration period. After the initial period of equilibration of tritium or deuterium, the slope gradually adopts a single exponential, that of total hydrogen turnover. This gradual decrement in concentration does not enter into the calculation of early equilibration.

Repeated Measurements

The isotope-dilution technique for measuring TBW is highly reproducible. In five repeated measurements in three dogs at weekly intervals, Moore et al. found small differences that approximated the expected statistical errors due to counting and chemistry (26).

In six normal human subjects studied, in which TBW was measured on two different occasions with an interval of 7-155 days, the measured TBW was constant to within 2.0% of the body weight, despite weight changes as large as 3.7 kg. The small variations detected could, again, be ascribed either to technical errors of small magnitude or a change in body water content during the period intervening between the determinations (10).

Carcass Desiccation

Turning now to the direct validation of total water methods by carcass desiccation, the following are re-

![Graph 1: Analysis of variance of total body water in the rat measured by desiccation versus body weight.]

![Graph 2: Analysis of variance of total body water in the rat measured by tritium dilution versus body weight.]

![Graph 3: Covariance analysis of desiccation versus tritium dilution in the rat.]

During this time isotope concentration decreases in an exponential fashion, until equilibration concentration is attained, generally within 1-2 h (6). It has been shown by in vitro studies that the membrane of erythrocytes presents a minimal barrier to isotope uptake (15).

An explanation of the delay of reaching an equilibrium in isotope distribution, despite its rapid diffusion, has been attributed to the existence of low flow and large total volume of certain regions of the body (6). Edelman and Moore (11) calculated that the extracellular water exchanges with cell water at the rate of 21% per minute in humans and 25% per minute in dogs. The long term arterial time-concentration curve of heavy water injection has three phases: its early slopes are determined by cell permeability, its equilibrium value by total body water and its later slope by water turnover rates (biological decay) (11).

Validation of the method for total body water may be found in three lines of investigation: internal consistency of derived values, repeated measurements under constant conditions in man or animals demonstrating reproducibility, and direct check in the same animal by cadaver desiccation (discussed herein).
viewed briefly: 1) previous data in the literature; 2) data from this study as compared with those of Huggins' laboratory; and 3) protein hydrogen exchangeability, in the light of these findings.

Previous data in the literature. Values ranging from 63.9 to 74% have been reported as estimates of TBW in the rat by body desiccation (1, 9, 14, 18, 35), Other species (cat, rabbit, guinea pig, and dog) showed values within the same limits (12, 14, 19, 26, 36). Body water measurements by tritium dilution in the rat ranged between 68.1 and 74.4% (14, 35). A summary of water measurements by tritium dilution in the rat within the same limits (12, 14, 19, 23, 26, 36).

Table 2. Summarizing data from literature on body water information from animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Wt, g</th>
<th>TBW Desiccation, %</th>
<th>TBW Dilution, %</th>
<th>Author and Year</th>
</tr>
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<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>112-126</td>
<td></td>
<td>66.5</td>
<td></td>
<td>Donaldson, 1915</td>
</tr>
<tr>
<td>34-295</td>
<td></td>
<td>57.6-68.7 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150-250</td>
<td>63.9 ± 1.3 (18)</td>
<td>68.1 ± 1.2 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>165 ± 3</td>
<td>66.5 ± 0.8 (7)</td>
<td>70.6 ± 1.3 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>227 ± 3</td>
<td>68.1 ± 0.9 (22)</td>
<td>74.4 ± 1.18 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td>64.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>68.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea</td>
<td>300-420</td>
<td>73.6 ± 1.6 (9)</td>
<td>72.8 ± 3.3 (9)</td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>65.7</td>
<td>55.5 ± 2.3 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>18,850± 2,542</td>
<td>64.4 ± 6.2 (5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate number of animals studied. * Rats studied following a nitrogen-free diet.

Table 3. Summarizing data from literature on body water information from man at different ages

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Wt, kg</th>
<th>Desiccation, %</th>
<th>Dilution, %</th>
<th>Author and Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td></td>
<td>66.4</td>
<td>Bischoff, 1963</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>74.1</td>
<td>Fehling, 1939</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>75.5</td>
<td>Iob, 1934</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>65.8</td>
<td>Widdowson, 1951</td>
<td></td>
</tr>
<tr>
<td></td>
<td>69.2 ± 7.0 (6)</td>
<td>73.0</td>
<td>Camerer, 1902</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>53.8</td>
<td></td>
<td>Widdowson, 1951</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>63</td>
<td></td>
<td>Bischoff, 1963</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>65.7</td>
<td></td>
<td>Volkman, 1881</td>
<td></td>
</tr>
<tr>
<td>35*</td>
<td>67.9</td>
<td></td>
<td>Mitchell, 1945</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>58.5</td>
<td></td>
<td>Bischoff, 1963</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>61.8</td>
<td></td>
<td>Widdowson, 1951</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>56.0</td>
<td></td>
<td>Widdowson, 1951</td>
<td></td>
</tr>
<tr>
<td>48*</td>
<td>81.5</td>
<td></td>
<td>Widdowson, 1951</td>
<td></td>
</tr>
<tr>
<td>67±</td>
<td>43.4</td>
<td>73.7</td>
<td>Moore, 1968</td>
<td></td>
</tr>
<tr>
<td>23-54±</td>
<td>72.5</td>
<td>54.3 (10)</td>
<td>Moore, 1963</td>
<td></td>
</tr>
<tr>
<td>71-94±</td>
<td>66.1</td>
<td>50.6 (7)</td>
<td>Moore, 1963</td>
<td></td>
</tr>
<tr>
<td>23-51±</td>
<td>59.3</td>
<td>48.6 (10)</td>
<td>Moore, 1963</td>
<td></td>
</tr>
<tr>
<td>60-74±</td>
<td>63.9</td>
<td>43.4 (7)</td>
<td>Moore, 1963</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate number of subjects studied. * Edematous subject. † Terminal carcinoma, 1-2 min interval between measurements. ‡ Normal series.

Table 4. Total body water of rabbits (22)

<table>
<thead>
<tr>
<th>Expt No</th>
<th>Live Wt, g</th>
<th>% Body wt</th>
<th>Direct</th>
<th>D2O space</th>
<th>Deviation</th>
<th>% Body wt</th>
<th>Direct</th>
<th>D2O space</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,888.0</td>
<td>72.5</td>
<td>67.6</td>
<td>-4.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>682.5</td>
<td>72.8</td>
<td>72.6</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1,293.0</td>
<td>72.6</td>
<td>71.8</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>765.5</td>
<td>72.0</td>
<td>75.4</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>510.0</td>
<td>71.4</td>
<td>73.0</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1,769.0</td>
<td>73.8</td>
<td>74.4</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1,470.0</td>
<td>75.5</td>
<td>72.9</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1,298.0</td>
<td>75.8</td>
<td>76.2</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1,235.8</td>
<td>75.0</td>
<td>68.4</td>
<td>-6.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ±SD 1,007.9 ± 986.6 73.6 ± 72.8 -0.78 ± 0.400.0 1.6 ± 3.3 3.5

interval between measurements. This unique study of cadaver analysis corroborated the data obtained by isotope dilution during life within the limits imposed by difficult clinical conditions.

To test the hypothesis that TBW measured by isotope dilution rendered true value of TBW, Moore (23) measured TBW by isotope dilution in rabbits. The isotope used at that time was deuterium. Afterward the rabbits were subjected to desiccation using rigorous techniques to rid the carcass of all water. By the weight differences, the TBW in the rabbits was measured directly. This figure was then compared with the total body water as measured by deuterium dilution prior to death (see Table 4). It was concluded then that the deuterium space constituted an accurate measurement of TBW (23). The results obtained by us in the present series corroborate this conclusion.

Data from Huggins' laboratories; relation to the findings reported herein. The data reported by Tisavipat et al. from Huggins' laboratories (35) (see Table 2 and Fig. 4) show a mean value for total body water by deuterium dilution prior to death (see Table 4). It was concluded then that the deuterium space constituted an accurate measurement of TBW (23). The results obtained by us in the present series corroborate this conclusion.

From the work of Sheng and Huggins (32) on total body water in the beagle, interpretive comment is more difficult. In Table 1 in the paper by Sheng and Huggins it is quite evident that there are very large discrepancies between desiccation and dilution values. For example, several of the animals show desiccation values in the region of 46.5-61.3% of body weight (very low for any living organism unless excessively obese), while in those same animals the tritium dilution data may run from 76 to 88.9% of body weight, excessively high for total body water in any living species and only associ-
Errors in isotope-dilution methods are usually up-
to 10.2% by 10.2% on July 1, 2017 http://ajpregu.physiology.org/ Downloaded from
ated with almost total loss of body fat. One can only
conclude that the data on the beagle were obtained
under difficult circumstances in which either the desic-
cation procedures or the tritium-dilution methods, or
both, were grossly in error.

Errors in isotope-dilution methods are usually up-
ward and nonrandom. Any loss of isotope in tritium-
dilution technique produces a falsely elevated value. By
contrast, in desiccation, any error due to incompleteness
in drying, produces a false low value for water content.
Compounding of these two potential errors widens the
gap between the isotope-dilution and the desiccation
values.

It should be stressed that in our desiccation methods
all bone and viscera were tediously cut into multiple 1-
cm³ pieces, taking special care to break all bones, to
expose marrow content and the contents of the cranial
cavity, ensuring adequate evaporation of all body wa-
ter. At the temperature used for desiccation no charring
carcass protein was observed.

Protein hydrogen exchangeability. These data, as an
incidental finding suggest that much of the hydrogen in
protein potentially available for immediate exchange
(7) does not exchange.

Interpreting our findings in the light of Blout et al.'s
work (3), it appears that in living man the steric confor-
mations of most of the proteins are such as to render the
potentially exchangeable hydrogens unavailable for im-
mediate exchange. In the terms of Blout et al. there
must be a large fraction of potentially exchangeable
protein hydrogen in the "hard-to-exchange amide hy-
drogen" (HEAH). This conclusion is offered because the
systematic error between the two methods is only 1 part
in 70 or approximately 1.7% of the total body water. As
indicated in the prior paper of this series (7), the error
that would be introduced into the method, were all the
protein hydrogens to be exchangeable, would be as a
maximum value 5.2% of the total body water. Thus, it
appears that protein hydrogen exchange is but a minor
feature of the early equilibrium phenomenon of tritium
dilution. It also appears that the large systematic error
reported by Huggins et al. must be traceable to some
other methodological disparity, rather than the identifi-
cation of an unexpectedly large amount of nonaqueous
exchangeable hydrogen.

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