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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CULEBRAS, JESUS M., GARRY F. FITZPATRICK, MURRAY F. BRENNAN, CARYL M. BOYDEN, AND FRANCIS D. MOORE. 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. Am. J. Physiol. 232(1): R60-R65, 1977 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 1(1): R60-R65, 1977. -Total body water (TBW) determination by tritium space could be factiously elevated by exchangeable H+ contained within water-soluble chemical configurations. Should this nonaqueous (molecular) exchangeable H+ turn out to be a large fraction of total exchangeable H+, TBW measurement by tritiated water (THO) dilution would display a systematic upward and non-random error. TBW was measured by THO dilution and subsequently by total body desiccation in 21 rats (weight 227 ± 83 g, mean ± SD). TBW was 71.38 ± 2.4% by THO dilution and 70.20 ± 1.5% by body desiccation. Analysis of variance of TBW vs. body weight showed a highly significant correlation both with desiccation (P < 0.0005, r = -0.78) and dilution (P < 0.03, r = -0.50). Covariance analysis of both methods showed no difference in slope (P > 0.9). There was a difference in variance (P < 0.001) and means (P < 0.03). Tritium space is 1.2% of body weight larger than TBW measured by desiccation. TBW measured by THO dilution gives a 1.71% overestimation of TBW as measured by desiccation. TBW measurement by THO dilution is accurate within < 2% error. These findings have particular significance in the light of our theoretical model of the total nonaqueous exchangeable H+ in fat, protein, and carbohydrate in the living vertebrate.

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 THO was made intraperitoneally with the rat under ether anesthesia. The average amount of THO injected was 1.0247 ± 0.004 ml. The injection was made with a 1 ml disposable plastic tuberculin syringe with a 27-gauge ½ needle. The syringe 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

ISOTOPE-DILUTION METHODS are used to quantify the total volume of exchangeable substances in the body. A small tracer dose is injected; equilibrium is allowed to proceed; the isotope-dilution equation, corrected for excretion or metabolic turnover, permits calculation of the total exchangeable element from the specific activity at equilibrium. We are concerned here with the measurement of the total exchangeable hydrogen in the body, expressed as water volume. In any isotope-dilution method, direct biological validation is essential in establishing reliability.

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.2

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 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 was also measured by weight differences and analyzed for tritium content to make adequate corrections.

The equilibrium tritium concentration in serum was measured by counting in a Nuclear Chicago liquid scintillation counter. The serum was prepared for counting by mixing 0.2 ml with 10 ml of a standard phosphor solution. Finally, correction was made for the solids in serum as already described (27). All counts were carried to a total of 60,000, usually requiring about 10 minutes for each sample.

RESULTS3

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 0.99% of total body weight. After correction for 0.99% water evaporation, and that its THO concentration was at least 10,000, each sample.

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).

Table 1. Total body water in rat

<table>
<thead>
<tr>
<th>Expt No</th>
<th>Live Wt, g</th>
<th>Total Body Water g or ml</th>
<th>% Body Wt</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Direct (desiccation)</td>
<td>Indirect (dilution)</td>
<td>Direct (desiccation)</td>
</tr>
<tr>
<td>1</td>
<td>395.7</td>
<td>270.2</td>
<td>268.6</td>
<td>68.28</td>
</tr>
<tr>
<td>2</td>
<td>384.0</td>
<td>282.5</td>
<td>284.7</td>
<td>63.78</td>
</tr>
<tr>
<td>3</td>
<td>161.2</td>
<td>111.9</td>
<td>112.3</td>
<td>70.05</td>
</tr>
<tr>
<td>4</td>
<td>143.1</td>
<td>102.3</td>
<td>102.4</td>
<td>71.48</td>
</tr>
<tr>
<td>5</td>
<td>158.5</td>
<td>111.8</td>
<td>112.7</td>
<td>70.52</td>
</tr>
<tr>
<td>6</td>
<td>156.8</td>
<td>110.6</td>
<td>111.1</td>
<td>70.51</td>
</tr>
<tr>
<td>7</td>
<td>160.2</td>
<td>112.4</td>
<td>114.4</td>
<td>70.18</td>
</tr>
<tr>
<td>8</td>
<td>191.1</td>
<td>137.3</td>
<td>140.4</td>
<td>71.87</td>
</tr>
<tr>
<td>9</td>
<td>220.9</td>
<td>156.7</td>
<td>160.7</td>
<td>70.95</td>
</tr>
<tr>
<td>10</td>
<td>209.7</td>
<td>150.8</td>
<td>152.9</td>
<td>71.90</td>
</tr>
<tr>
<td>11</td>
<td>256.6</td>
<td>180.5</td>
<td>186.5</td>
<td>70.36</td>
</tr>
<tr>
<td>12</td>
<td>205.4</td>
<td>146.0</td>
<td>139.9</td>
<td>71.10</td>
</tr>
<tr>
<td>13</td>
<td>221.1</td>
<td>152.5</td>
<td>156.9</td>
<td>68.99</td>
</tr>
<tr>
<td>14</td>
<td>219.4</td>
<td>153.5</td>
<td>152.2</td>
<td>69.95</td>
</tr>
<tr>
<td>15</td>
<td>198.1</td>
<td>141.7</td>
<td>140.2</td>
<td>71.51</td>
</tr>
<tr>
<td>16</td>
<td>269.4</td>
<td>170.8</td>
<td>185.7</td>
<td>69.30</td>
</tr>
<tr>
<td>17</td>
<td>251.5</td>
<td>171.6</td>
<td>172.1</td>
<td>68.22</td>
</tr>
<tr>
<td>18</td>
<td>140.6</td>
<td>130.1</td>
<td>107.9</td>
<td>73.40</td>
</tr>
<tr>
<td>19</td>
<td>196.5</td>
<td>139.9</td>
<td>145.5</td>
<td>70.47</td>
</tr>
<tr>
<td>20</td>
<td>230.5</td>
<td>161.5</td>
<td>170.6</td>
<td>70.06</td>
</tr>
<tr>
<td>21</td>
<td>307.0</td>
<td>209.5</td>
<td>219.8</td>
<td>68.24</td>
</tr>
</tbody>
</table>

Mean 227.11 158.49 161.13 70.20 71.38 1.19 ±SD 83.7 54.6 55.1 1.5 2.44 1.77 ±SE 18.3 11.9 12.0 0.3 0.53 0.4

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 equilibration 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).

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 < 0.03) (Fig. 3).

DISCUSSION

These rather elaborate precautions, carried out to correct for water and solids in the withdrawn blood, or that lost by syringe handling, are mentioned here in some detail. Although they are very small, their omission could account for some random systematic errors in total body water determination.

All results expressed as mean ± SD, unless otherwise stated.

A 0.90% insensible weight loss during the period of 2 h between THO injection and killing the rat must be introduced in the calculation. We have assumed that this weight loss was entirely due to water evaporation, and that its THO concentration was at least equal to that at equilibrium concentration.
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).

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).

Repeated Measurements

The isotope-dilution technique for measuring THW 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-
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, 23, 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 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, 23, 26, 36).

In Table 2 data reported in the literature can be found. A summary of water information from man at different ages is shown (2, 4, 13, 17, 22, 27, 25, 37, 38).

A body compositional study was performed by Moore et al. (25) in a patient with incurable cancer. The reconciliation between total body water measured by THO dilution and cadaver analysis showed only a difference of 5 ml. Correction for weight variations were done to account for the changes occurring during the month.

In Table 3 data on TBW measurements in man under various circumstances and ages is shown (2, 4, 13, 17, 22, 27, 25, 37, 38).

**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>
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<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>112-126</td>
<td>65.9</td>
<td></td>
<td></td>
<td>Donaldson, 1915</td>
</tr>
<tr>
<td>112-126</td>
<td>66.7</td>
<td></td>
<td></td>
<td>Light, 1943</td>
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<tr>
<td>34-285</td>
<td>57.6</td>
<td>65.7</td>
<td></td>
<td>Aschworth, 1938</td>
</tr>
<tr>
<td>150-250</td>
<td>63.0</td>
<td>70.0</td>
<td></td>
<td>Ashwood, 1890</td>
</tr>
<tr>
<td>150-250</td>
<td>66.1</td>
<td>66.5</td>
<td></td>
<td>Light, 1943</td>
</tr>
<tr>
<td>100-150</td>
<td>60.0</td>
<td>60.0</td>
<td></td>
<td>Foy, 1960</td>
</tr>
<tr>
<td>227-340</td>
<td>68.9</td>
<td>70.2</td>
<td></td>
<td>=Cudner, 1970</td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>64.2</td>
<td></td>
<td></td>
<td>Foy, 1960</td>
</tr>
<tr>
<td>68.8</td>
<td></td>
<td></td>
<td></td>
<td>Krause, 1884</td>
</tr>
<tr>
<td>1,000-2,000</td>
<td>76.3</td>
<td>73.6</td>
<td>66.6</td>
<td>Foy, 1960</td>
</tr>
<tr>
<td>Guinea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100-420</td>
<td>75.0</td>
<td>75.0</td>
<td></td>
<td>Moore, 1927</td>
</tr>
<tr>
<td>18,820</td>
<td>65.7</td>
<td>66.8</td>
<td></td>
<td>Engels, 1894</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>
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</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>66.4</td>
<td>65.0</td>
<td>65.0</td>
<td>Bischoff, 1963</td>
</tr>
<tr>
<td>Adult</td>
<td>68.2</td>
<td>68.2</td>
<td>68.2</td>
<td>Fehling, 1939</td>
</tr>
<tr>
<td>Adult</td>
<td>68.8</td>
<td>75.5</td>
<td>75.5</td>
<td>Iob, 1930</td>
</tr>
<tr>
<td>35*</td>
<td>68.8</td>
<td>68.8</td>
<td>68.8</td>
<td>Widdowson, 1915</td>
</tr>
<tr>
<td>4.5</td>
<td>60.0</td>
<td>60.0</td>
<td>60.0</td>
<td>Camerer, 1902</td>
</tr>
<tr>
<td>Adult</td>
<td>63.0</td>
<td>63.0</td>
<td>63.0</td>
<td>Rischoff, 1963</td>
</tr>
<tr>
<td>Adult</td>
<td>65.7</td>
<td>65.7</td>
<td>65.7</td>
<td>Volkman, 1881</td>
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<tr>
<td>35*</td>
<td>67.9</td>
<td>67.9</td>
<td>67.9</td>
<td>Mitchell, 1945</td>
</tr>
<tr>
<td>35*</td>
<td>58.5</td>
<td>58.5</td>
<td>58.5</td>
<td>Bischoff, 1963</td>
</tr>
<tr>
<td>35*</td>
<td>61.8</td>
<td>61.8</td>
<td>61.8</td>
<td>Widdowson, 1915</td>
</tr>
<tr>
<td>42*</td>
<td>56.0</td>
<td>56.0</td>
<td>56.0</td>
<td>Widdowson, 1915</td>
</tr>
<tr>
<td>48*</td>
<td>81.5</td>
<td>81.5</td>
<td>81.5</td>
<td>Widdowson, 1915</td>
</tr>
<tr>
<td>67*</td>
<td>53.8</td>
<td>53.8</td>
<td>53.8</td>
<td>Moore, 1927</td>
</tr>
<tr>
<td>67*</td>
<td>53.8</td>
<td>53.8</td>
<td>53.8</td>
<td>Moore, 1927</td>
</tr>
<tr>
<td>67*</td>
<td>53.8</td>
<td>53.8</td>
<td>53.8</td>
<td>Moore, 1927</td>
</tr>
</tbody>
</table>

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

**TABLE 4. Total body water of rabbits (22)**

<table>
<thead>
<tr>
<th>Ext No.</th>
<th>Live Wt, g</th>
<th>g or ml</th>
<th>% Body wt</th>
<th>Deviation ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Direct D2O space</td>
<td>Direct D2O space</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2,888.0</td>
<td>2,095.5</td>
<td>1,950.0</td>
<td>72.5</td>
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<tr>
<td>2</td>
<td>862.5</td>
<td>630.5</td>
<td>626.0</td>
<td>73.1</td>
</tr>
<tr>
<td>3</td>
<td>1,129.0</td>
<td>825.0</td>
<td>806.0</td>
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<tr>
<td>4</td>
<td>753.5</td>
<td>542.9</td>
<td>559.0</td>
<td>72.0</td>
</tr>
<tr>
<td>5</td>
<td>510.0</td>
<td>600.5</td>
<td>684.0</td>
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<td>6</td>
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<td>1,398.0</td>
<td>1,370.0</td>
<td>73.8</td>
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<td>7</td>
<td>1,470.0</td>
<td>1,110.0</td>
<td>1,071.0</td>
<td>75.5</td>
</tr>
<tr>
<td>8</td>
<td>1,290.0</td>
<td>983.5</td>
<td>989.0</td>
<td>75.8</td>
</tr>
<tr>
<td>9</td>
<td>1,235.8</td>
<td>926.8</td>
<td>846.0</td>
<td>75.0</td>
</tr>
</tbody>
</table>

Mean 1,385.3 1,007.9 966.6 73.6 72.8 0.78

To test the hypothesis that TBW measured by isotope dilution rendered true value of TBW, Moore (23) measured TBW by isotope dilution in rabbits. 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 carcasses 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 tritium dilution that is 74.4% of body weight in the rat. This is 3% higher than the value that we report herein. In addition, their values obtained by desiccation suggest the possibility of incomplete drying since the values for total body water are 66.4% of body weight or 3.8% lower than ours. Taking the discrepancy between the two methods (tritium dilution versus desiccation) therefore, a large discrepancy appears in Tisavipat and Huggins' work, amounting to about 12% of the total body water.

By comparison of our results reported herein with those of Foy and Schneider (14) (Table 2 and Fig. 4) the tritium values are almost identical, whereas both series reported by Foy and Schneider show total body waters by desiccation considerably lower than our findings.

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-
ward and nonrandom. Any loss of isotope in tritium-dilution methods, or the tritium-dilution methods, or the desiccation procedures or the tritium-dilution methods, or under difficult circumstances in which either the desiccation or the tritium-dilution methods, or both, were grossly in error.

Errors in isotope-dilution methods are usually up to 10.2% on October 22, 2017 http://ajpregu.physiology.org/ Downloaded from

FIG. 4. Total body water measured by isotope dilution and checked by carcass desiccation in the rat. Comparison of results obtained in this experiment with others reported in literature.

raped 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 desiccation procedures or the tritium-dilution methods, or both, were grossly in error.

Errors in isotope-dilution methods are usually upward 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 water. At the temperature used for desiccation no charring of 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 conformations of most of the proteins are such as to render the potentially exchangeable hydrogens unavailable for immediate 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 hydrogen" (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 identification of an unexpectedly large amount of nonaqueous exchangeable hydrogen.

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