Body Fluid Dynamics: Back to the Future

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ABSTRACT

Pioneering investigations conducted over a half century ago on tonicity, transcapillary fluid exchange, and the distribution of water and solute serve as a foundation for understanding the physiology of body fluid spaces. With passage of time, however, some of these concepts have lost their connectivity to more contemporary information. Here we examine the physical forces determining the compartmentalization of body fluid and its movement across capillary and cell membrane barriers, drawing particular attention to the interstitium operating as a dynamic interface for water and solute distribution rather than as a static reservoir. Newer work now supports an evolving model of body fluid dynamics that integrates exchangeable Na\(^+\) stores and transcapillary dynamics with advances in interstitial matrix biology.


Compartmentalization of Body Water

The content of total body water (TBW) is a physiologic function of tissue composition leading to qualitatively predictable alterations with age, gender, and body weight. Most tissues such as skin, muscle, visceral organs, and brain consist of 70 to 80% water by weight, whereas adipose tissue and bone are only 10 to 20% water. TBW reflects a weighted average of tissue water content with relatively lower values in subjects with greater adiposity or lower muscle mass. Relative to weight, women and elderly individuals generally have less body water because of higher content of body fat or preferential loss of muscle mass with age, respectively. TBW increases with obesity but decreases relative to body weight with the gain of relatively drier adipose tissue.\(^1\)\(^2\)

Nephrologists routinely estimate TBW to gauge electrolyte and fluid deficits with hypovolemia or hypertonicity, assess dialytic adequacy using TBW as a surrogate for the volume of distribution of urea, guide drug dosing, and rationalize dialytic clearance of toxins. TBW is classically estimated as 60% of body weight in men and 50% of body weight in women deducting 5% for elderly patients.\(^3\) Given physiologic variation in body tissue composition, early investigators recognized that absolute weight-based rules of estimation apply only to a select population of healthy individuals\(^4\) and subsequently derived regression equations better predict TBW in a broader range of subjects. Anthropomorphic equations including age, gender, ethnicity, weight, and height are now available to im-
Body fluid compartments. In the “average” adult man, intracellular fluid (ICF) and extracellular fluid (ECF) domains consist of about 57% and 43% of total body water (TBW). The ECF compartment is further subdivided into interstitial fluid (ISF)/lymph, plasma, bone and connective tissue, adipose tissue, and transcellular water. Skeletal muscle predominates the ICF. Percentages are percent of TBW. Adapted from references 2 and 27. RBCs, red blood cells.

The ECF compartment is further subdivided into interstitial fluid (ISF)/lymph, plasma, bone and connective tissue, adipose tissue, and transcellular water. Delineating the exact distribution of ECF among its compartments requires an integration of classic tracer distribution volumes with anatomic data including classic human dissection and chemical tissue analysis or more recent imaging techniques. Consistent estimates of plasma, interstitial, transcellular, and adipose ECF volumes have been delineated despite varying data sources over time (Figure 1). Dense connective tissue and bone pose technical difficulties, particularly with the accurate separation of connective tissue, muscle, and marrow from bone, variation in bone preparation (± marrow, ± articular cartilage, cortical, or trabecular bone), and significant loss of water with age. Edelman and Leibman drew upon historic dissection data for skeletal water as 16% of body weight, skeletal water content as 25 to 30% by weight, and assumed skeletal water is >90% extracellular to calculate ECF bone water as 7.5% of TBW. Unfortunately, skeletal water is not >90% extracellular, because skeletal weight includes bone and enclosed marrow (Table 2). Normally active bone marrow is highly cellular, predominantly intracellular water. When bone and marrow are accounted for, skeletal water is only about 60% extracellular, reducing bone ECF to 3% of TBW (Table 2).

Skeletal muscle water is often underappreciated but accounts for 40 to 50% of TBW, nearly 75% of ICF and cell mass, and about 33% of interstitial fluid volume. Loss of muscle mass redistributes TBW from ICF to ECF and increases the ratio of plasma to interstitial fluid volume. To clarify the compartmentalization of body water, we provide a balance sheet for body weight and water distribution among the major body tissues for men and women (Table 3).

Forces Governing Water Distribution

Hydrostatic pressure and osmosis drive water between the ECF and ICF compartments, and the eventual body water distribution reflects a steady state of these forces:

\[ P_{ec} - P_{ic} = \Pi_{ic} - \Pi_{ec} \quad (Eq. 1) \]

\[ P_{ic} \] and \[ P_{ec} \] represent intracellular and extracellular hydrostatic pressures, respectively, and \[ \Pi_{ic} \] and \[ \Pi_{ec} \] represent osmotic pressures.

Table 1. Tracer distributions for measurement of ECF volume

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Volume of Distribution (% TBW)</th>
<th>Plasma/Lymph/Interstitium</th>
<th>Connective Tissue</th>
<th>Bone</th>
<th>ICF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inulin</td>
<td>25 to 33</td>
<td>+++</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>sucrose</td>
<td>30 to 36</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>mannitol</td>
<td>33 to 39</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>glucose</td>
<td>42 to 46</td>
<td>+++</td>
<td>+ + +</td>
<td>(+10%)</td>
<td>0</td>
</tr>
<tr>
<td>Sulfate</td>
<td>33 to 39</td>
<td>+++</td>
<td>+ + +</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloride (or bromide)</td>
<td>46 to 52</td>
<td>+++</td>
<td>+ + +</td>
<td>(+10%)</td>
<td>0</td>
</tr>
<tr>
<td>Sodium</td>
<td>50 to 55</td>
<td>+++</td>
<td>+ + +</td>
<td>0</td>
<td>(+3%)</td>
</tr>
</tbody>
</table>

0, <5%; +, 5 to 35%; ++, 35 to 75%; ++++, 75 to 125%; ++++, >125% penetration. Adapted from references 2,11–15, and 182. TBW, total body water; ICF, intracellular fluid; ECF, extracellular fluid.
motic pressures. Plants, fungi, and bacteria possess rigid cell walls that generate hydrostatic pressure to counteract osmotic pressure gradients. Animal cells shed these reinforced walls during evolution to gain flexibility, but the cost of this loss leaves cell volume regulation to the mercy of extracellular tonicity29,30.

Table 2. Distribution of water and Na\textsuperscript{+} within bone and marrow (skeleton)

<table>
<thead>
<tr>
<th>Skeletal Component</th>
<th>Water Content (L)</th>
<th>Relative Water (% of Tissue Mass)</th>
<th>ECF Water (L)</th>
<th>Relative ECF (% of TBW)</th>
<th>Na\textsuperscript{+} Content (mEq/kg of body weight)</th>
<th>Tissue [Na\textsuperscript{+}] (mEq/L H\textsubscript{2}O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>0.9</td>
<td>15</td>
<td>0.9</td>
<td>2.1</td>
<td>18 to 20 (5)</td>
<td>-1500 (~400)</td>
</tr>
<tr>
<td>Active marrow</td>
<td>0.9</td>
<td>80</td>
<td>0.18</td>
<td>0.4</td>
<td>0.4</td>
<td>30</td>
</tr>
<tr>
<td>Inactive marrow</td>
<td>0.4</td>
<td>15</td>
<td>0.32</td>
<td>0.75</td>
<td>0.6</td>
<td>110</td>
</tr>
<tr>
<td>Total</td>
<td>2.2</td>
<td>23</td>
<td>1.4</td>
<td>3.25</td>
<td>21 (6)</td>
<td>700 (200)</td>
</tr>
</tbody>
</table>

Tissue water (%) 63

The values are estimates for average adult man. The values in parentheses represent exchangeable Na\textsuperscript{+} as opposed to total Na\textsuperscript{+}. Based on references\textsuperscript{10,14,18,25,26,183. TBW, total body water; ECF, extracellular fluid.}

Table 3. Body water distribution among tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Weight (kg)</th>
<th>Body Weight (%)</th>
<th>Water Content Fraction</th>
<th>ECF Fraction</th>
<th>ECF Water (L)</th>
<th>ICF Water (L)</th>
<th>Total Water (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>5.5</td>
<td>7.53</td>
<td>0.80</td>
<td>0.63</td>
<td>2.79</td>
<td>1.61</td>
<td>4.40</td>
</tr>
<tr>
<td>woman</td>
<td>4.1</td>
<td>6.83</td>
<td>0.80</td>
<td>0.68</td>
<td>2.23</td>
<td>1.05</td>
<td>3.28</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>28.5</td>
<td>39.04</td>
<td>0.76</td>
<td>0.16</td>
<td>3.47</td>
<td>18.19</td>
<td>21.66</td>
</tr>
<tr>
<td>woman</td>
<td>18</td>
<td>30</td>
<td>0.76</td>
<td>0.16</td>
<td>2.19</td>
<td>11.49</td>
<td>13.68</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>4.3</td>
<td>5.89</td>
<td>0.72</td>
<td>0.95</td>
<td>2.94</td>
<td>0.15</td>
<td>3.10</td>
</tr>
<tr>
<td>woman</td>
<td>3</td>
<td>5</td>
<td>0.72</td>
<td>0.95</td>
<td>2.05</td>
<td>0.11</td>
<td>2.16</td>
</tr>
<tr>
<td>Brain and viscera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>6</td>
<td>8.22</td>
<td>0.72</td>
<td>0.35</td>
<td>1.51</td>
<td>2.81</td>
<td>4.32</td>
</tr>
<tr>
<td>woman</td>
<td>5.4</td>
<td>9</td>
<td>0.72</td>
<td>0.35</td>
<td>1.36</td>
<td>2.53</td>
<td>3.89</td>
</tr>
<tr>
<td>Bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>5.75</td>
<td>7.88</td>
<td>0.15</td>
<td>1.00</td>
<td>0.86</td>
<td>0.00</td>
<td>0.86</td>
</tr>
<tr>
<td>woman</td>
<td>4.2</td>
<td>7</td>
<td>0.15</td>
<td>1.00</td>
<td>0.63</td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>Active Marrow</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>1.17</td>
<td>1.6</td>
<td>0.80</td>
<td>0.20</td>
<td>0.19</td>
<td>0.75</td>
<td>0.94</td>
</tr>
<tr>
<td>woman</td>
<td>0.9</td>
<td>1.5</td>
<td>0.80</td>
<td>0.20</td>
<td>0.14</td>
<td>0.58</td>
<td>0.72</td>
</tr>
<tr>
<td>Inactive Marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>2.48</td>
<td>3.4</td>
<td>0.15</td>
<td>0.80</td>
<td>0.30</td>
<td>0.07</td>
<td>0.37</td>
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<tr>
<td>woman</td>
<td>1.8</td>
<td>3</td>
<td>0.15</td>
<td>0.80</td>
<td>0.22</td>
<td>0.05</td>
<td>0.27</td>
</tr>
<tr>
<td>Connective Tissue</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>3.7</td>
<td>5.07</td>
<td>0.80</td>
<td>1.00</td>
<td>2.96</td>
<td>0.00</td>
<td>2.96</td>
</tr>
<tr>
<td>woman</td>
<td>3</td>
<td>5</td>
<td>0.80</td>
<td>1.00</td>
<td>2.4</td>
<td>0.00</td>
<td>2.4</td>
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<tr>
<td>Transcellular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>1.1</td>
<td>1.51</td>
<td>0.95</td>
<td>1.00</td>
<td>1.05</td>
<td>0.00</td>
<td>1.05</td>
</tr>
<tr>
<td>woman</td>
<td>1.1</td>
<td>1.83</td>
<td>0.95</td>
<td>1.00</td>
<td>1.05</td>
<td>0.00</td>
<td>1.05</td>
</tr>
<tr>
<td>Adipose Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>man</td>
<td>14.5</td>
<td>19.86</td>
<td>0.14</td>
<td>0.80</td>
<td>1.62</td>
<td>0.41</td>
<td>2.03</td>
</tr>
<tr>
<td>woman</td>
<td>18.5</td>
<td>30.83</td>
<td>0.13</td>
<td>0.85</td>
<td>1.97</td>
<td>0.35</td>
<td>2.31</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17.69</td>
<td>24</td>
<td>41.68</td>
</tr>
</tbody>
</table>

% of TBW

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% of TBW</th>
<th>% of Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>man</td>
<td>73</td>
<td>57.1</td>
</tr>
<tr>
<td>woman</td>
<td>60</td>
<td>50.6</td>
</tr>
</tbody>
</table>

The values shown are for an average adult man or an average adult woman. Adapted from references\textsuperscript{1,2,4,10,14,18,25,26,184–186. TBW, total body water; ICF, intracellular fluid; ECF, extracellular fluid.}
Osmotic water movement is directly proportional to hydraulic permeability ($L_p$) and solute concentration gradient ($\Delta [c]$) in the ideal situation of an impermeable solute:

\[
alp{Osmotic Water Flux} = L_p \times \Delta \Pi_{\text{ideal}} = L_p \times 19.34 \times \varphi \times z \times \Delta [c] \tag{6}
\]

Most solutes are partially permeable and undergo convective transport along with water. Staverman introduced the reflection coefficient, $\sigma$, to relate the observed osmotic pressure gradient ($\Delta \Pi_{\text{obs}}$) to the ideal osmotic pressure gradient ($\Delta \Pi_{\text{ideal}}$) with an impermeable solute:

\[
\sigma = \frac{\Delta \Pi_{\text{obs}}}{\Delta \Pi_{\text{ideal}}} \tag{7}
\]

Osmotic Water Flux

\[
alp{Osmotic Water Flux} = L_p \times \sigma \times 19.34 \times \varphi \times z \times \Delta [c] \tag{8}
\]

$\sigma$ is a dimensionless number between 0 and 1. In the presence of a concentration gradient, a solute with $\sigma = 1$ produces maximal osmosis, whereas a solute with $\sigma = 0$ fails to generate osmotic water movement. Why is there no net water flux when $\sigma = 0$ despite a large concentration gradient ($\Delta [c] \gg 0$)? Qualitatively, solute movement down its concentration gradient provides free energy for water to move against its osmotic gradient, which counteracts favorable water movement in the opposite direction; thus, no net water transport results. For solute to provide free energy for uphill water movement, both solute and water must move in a coupled fashion along the same pathway.

Solute movement down its concentration gradient generates free energy for uphill water movement. Therefore, solute movement down its concentration gradient generates free energy for uphill water movement. Generally, solutes with low reflection coefficients ($\sigma \to 0$) have high $P_D$, because the transport pathway allowing for convective solute transport with water also typically supports diffusive solute movement. The converse is not necessarily true because solutes with high reflection coefficients ($\sigma \to 1$) may or may not exhibit low $P_D$, depending on the characteristics of the independent pathway for solute diffusion. If the independent pathway is simple diffusion through the lipid bilayer, $P_D$ is typically low. Alternatively, if the independent pathway is transporter facilitated, $P_D$ is relatively high. For example, along inner medullary collecting ducts, urea and water move independently through urea transporter and aquaporin facilitated pathways during high ADH states; thus, $P_D$ is high, but $\sigma$ is near 1.

Urea illustrates the relationship between solute osmotic efficacy and membrane permeability, diffusion surface area, and kinetics of solute generation or loss. Urea is relatively hydrophilic and exhibits low $P_D$ ($\approx 1$ to $5 \times 10^{-6}$ cm/s) and a reflection coefficient near 1 in artificial lipid bilayers; thus, urea is quite effective at eliciting osmosis when urea concentration gradients are abruptly created in these experimental systems. This is in contrast to ethanol, which exhibits high $P_D$ and a reflection coefficient near 0, even with model membranes. However, textbooks often suggest that urea is freely diffusible across membranes and therefore an ineffective solute. Urea transporters facilitate urea diffusion across some biologic membranes, but even the low permeability of pure lipid bilayers is sufficient to minimize urea concentration gradients as total cell membrane surface area is quite large ($\approx 1.2 \times 10^6$ cm$^2$ or 12,000 m$^2$), and urea generation rate is comparably meager. Even if one assumes a robust urea generation rate of about 40 g/d, pure lipid bilayer permeability, and no urea excretion or metabolism, a transcellular urea gradient of only about 0.025 mM is expected (see supplemental material). Transcapillary urea gradients are also minimal for most capillary beds as a result of high diffusive permeability and a low reflection coefficient ($\sigma < 0.1$) with urea easily traversing interendothelial pores. However, cerebrocapillaries exhibit low urea permeability with a sizeable reflection coefficient of about 0.5. If blood urea nitrogen falls at a rate of 50 mg/dl/h during hemodialysis, a cerebral transcapillary urea gradient of about 0.25 mM can develop leading to a $\approx 2.4$ mmHg (19.34 * 0.5 * 0.25 mOsm/kg) osmotic pressure favoring capillary filtration (see supplemental material). Cerebral interstitial edema or dialysis disequilibrium may ensue from such a rapid fall in blood urea nitrogen.

At the capillary-interstitial interface, plasma proteins are excluded from interendothelial pores and act as effective osmoles, whereas small solutes such as Na$^+$, Cl$^-$, and urea are ineffective osmoles freely moving across interendothelial spaces. Oncotic and colloid osmotic pressure (COP) are used to describe protein-generated osmotic pressure, but these pressures are not included in the transport of urine.
Inflammatory hypoalbuminemia behaves similarly as $\alpha_1$ and $\alpha_2$ fractions—the acute phase proteins—dramatically rise, but differs compared to analbuminemia because the $\beta$ fraction is unchanged, and the $\gamma$ fraction often increases with chronicity.\(^6^2\) Thus, globulin osmotic efficiency improves but slightly less compared with analbuminemia with a fall in globulin MW$_{av}$ from $\sim 150$ kD to $\sim 120$ kD (see supplemental material). The larger osmotic contribution of plasma globulins facilitates a robust defense of plasma COP in inflammatory states and explains the superior correlation of plasma total protein concentration with plasma COP compared with serum albumin in critically ill, hypoalbuminemic patients.\(^6^3\),\(^6^4\)

### Cell Volume Homeostasis and Body Na$^+$ and K$^+$ Distribution

Tonicity is shorthand for the action of effective osmolality across a barrier and in this context traditionally refers to the volume behavior of cells in a solution. Osmolality, on the other hand, measures both effective and ineffective osmoles in a kilogram of body fluid.\(^6^5\)–\(^6^7\) For instance, ethanol elevates plasma osmolality but does not affect tonicity by rapidly permeating lipid bilayers. Thus, estimates of tonicity are physiologically relevant, whereas osmolality is an imperfect surrogate for tonicity and requires appropriate discounting of ineffective osmoles.\(^6^5\)–\(^6^7\)

The relative abundance of effective osmoles in intracellular and extracellular compartments dictates body water distribution between ICF and ECF. All cells contain largely fixed or poorly permeable anions such as metabolites (ATP, phosphocreatine, and sulfate), nucleotides, and proteins.\(^6^3\) K$^+$ acts as the primary counter-ion and serves optimal ribosomal protein synthesis requiring high intracellular K$^+$ concentrations.\(^6^8\) The fixed intracellular anions and K$^+$ counter-ions create a Donnan effect-related osmotic gradient favoring persistent water entry. To counteract this osmotic gradient, Na$^+$–K$^+$-ATPases actively extrude Na$^+$ ions producing a double-Donnan effect.\(^6^9\) Cl$^-$ passively moves with Na$^+$ to maintain electroneutrality leading to osmotic equilibrium (Figure 2).\(^2^9\),\(^7^0\) In essence, the cells expend ATP to convert permeable Na$^+$ and K$^+$ ions into impermeable, effective osmoles sequestered in the ECF and ICF, respectively. Similarly, Cl$^-$ concentrates in the ECF, whereas fixed anions predominate in the ICF.\(^2^9\),\(^7^0\) Water passively distributes into the ECF or ICF compartments in proportion to the effective Na$^+$ and K$^+$ content to reach effective osmotic equilibrium (tonicity) and establish cell volume.

Almost 98% of total body Na$^+$ is distributed among the ECF subcompartments (Table 4). Total body Na$^+$ is often divided into exchangeable (Na$^+$$_{ex}$) and nonexchangeable domains on the basis of the extent of radioisotope Na$^+$ equilibration with the body pool. About 20 to 30% of total body Na$^+$ is nonexchangeable, residing in anhydrous bone matrix.\(^2^1\),\(^7^2\)–\(^7^4\) Total body K$^+$ diametrically mirrors Na$^+$ with about 95% located intracellularly (Table 4). Unlike Na$^+$, however, over 90% of body K$^+$ is exchangeable.\(^7^5\) To-

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**Table 4**

<table>
<thead>
<tr>
<th>Component</th>
<th>Exchangeable</th>
<th>Nonexchangeable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$$_{ex}$</td>
<td>0.7 mg/kg</td>
<td>0.2 mg/kg</td>
</tr>
<tr>
<td>K$^+$</td>
<td>0.2 mg/kg</td>
<td>0.0 mg/kg</td>
</tr>
</tbody>
</table>

**Figure 2**

Tonicity as a function of effective osmolality (tonicity) and establish cell volume.
toral body K⁺ is proportional to cell mass and reflects metabolic activity. Red blood cell volume supplies oxygen for tissue metabolism and expectedly correlates with total body 

**Figure 2.** Double-Donnan effect and cell volume homeostasis. (A) Fixed intracellular anions (A⁻) create a large Donnan effect-related osmotic pressure favoring untenable water entry. (B) The Na⁺/K⁺ ATPase essentially fixes Na⁺ ions extracellularly to create a Na⁺-related Donnan effect. The Na⁺ and fixed anion effects counteract one another to form a double-Donnan steady state with no transmembrane osmotic gradient. Adapted from reference 29.

**Table 4.** Body sodium and potassium distribution

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Interstitium</th>
<th>Bone and Connective Tissue</th>
<th>Intracellular</th>
<th>Total</th>
<th>Exchangeable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mEq/kg H₂O)</td>
<td>(mEq/kg H₂O)</td>
<td>(mEq/kg H₂O)</td>
<td>(mEq/kg H₂O)</td>
<td>(mEq/kg H₂O)</td>
<td>(mEq/kg)</td>
</tr>
<tr>
<td>Na⁺</td>
<td>6</td>
<td>150</td>
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<td>148.5</td>
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<td>K⁺</td>
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<td>4.2</td>
<td>0.5</td>
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<td>52.6 (49.3)</td>
<td>160 (150)</td>
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<td></td>
<td>56.6</td>
<td>53.3</td>
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</table>

The values are estimates for an average adult man. The parentheses indicate exchangeable cation opposed to total cation content and concentration. Adapted from references 2,14,183, and 187.

**Extracellular Fluid Dynamics**

New studies suggest that the plasma and lymphatic compartments and the interstitium are dynamic interfaces for water distribution. ECF is heterogeneous, yet plasma, representing approximately 17% of ECF, is used to assess body fluid homeostasis. Neurohormonal and renal homeostatic mechanisms sense and defend effective circulating blood volume, which primarily depends on lymphatic function.81 The modified Starling relationship mathematically delineates water flux between capillary plasma and interstitium36,45,83:

\[
\text{Net Capillary Filtration (J_v)} = \frac{P_c - P_l}{K_p}\]

(11) where \(P_c\) and \(P_l\) are capillary and interstitial hydrostatic pressures, \(P_c\) and \(P_l\) are capillary and interstitial colloid osmotic pressures, and \(K_p\) represents the osmotic reflection coefficient. At steady state, capillary filtration must equal lymphatic flow. The magnitude of hydrostatic and osmotic pressures and their balance across capillaries has been debated continuously over the last century. Physiology textbooks still present a framework where filtration predominates at the arterial end and reabsorption occurs at the venous end of the capillary with falling capillary hydrostatic pressure and constant capillary osmotic pressure (Figure 3).45 Recent studies, however, point to low net filtration with net filtration pressures (\(J_v/L_p\)) of 0.5 to 1 mmHg across the entire length of the capillary in most vascular beds (Figure 3).84 A better understanding of interstitial matrix and reassessment of \(P_c\) and \(P_l\) has driven this paradigm shift.

Interstitial and connective tissues are typically modeled as \(\text{triphasic systems}\)85: free flowing fluid with albumin, a gel phase with glycosaminoglycans (GAGs), and a collagen-based matrix (Figure 4). Water and small solutes (Na⁺, Cl⁻, and urea) move easily between all compartments according to prevailing osmotic, hydrostatic, and electrochemical forces.86 Albumin is excluded from the GAG compartment, and both GAGs and albumin are excluded from the collagen matrix.57,86 GAGs generate osmotic pressure (\(\Pi_{GAG}\)) both in proportion to concentration and negative charge density as the latter attracts cations.

\[\Delta \pi = \frac{RT}{V_f} \left( C_{GAG} - C_{GAG}^* \right) \]
Figure 3. Paradigm shift in transcapillary fluid exchange. (A and B) Classic view of transcapillary Starling forces with $\Pi_i$ and $P_i$ ignored leading to predominant filtration on the arterial end giving way to absorption on the venous end. (C and D) $\Pi_i$ is a nonlinear function of filtration rate ($J_v$). Filtration rate is the intersection of this function and $\Pi_i$ as a function of Starling forces. The Starling relationship is linear with a slope equal to $1/\sigma K_i$ and $y$-intercept of $\Pi_i + P_i/\alpha - P_c/\alpha$. As $P_c$ falls along the capillary, the linear Starling curve left shifts as the $y$-intercept increases. The left shift is blunted by a decrease in $P_i$. $\Pi_i$ increases with falling filtration, and the relative steepness of the nonlinear $\Pi_i(J_v)$ function maintains filtration along the capillary.

Figure 4. Triphasic interstitial model. Schematic representation of interstitial elements and their relationship to capillary forces and lymphatics. Interstitial $P_i$ is a balance between GAG osmotic pressure ($\Pi_{GAG}$) and collagen hydrostatic pressure ($P_{collagen}$). Net capillary filtration ($J_f$) must equal lymphatic flow ($J_L$) at steady state.

and increases tissue $Na^+$ concentration. The collagen matrix generates a hydrostatic pressure that typically opposes GAG osmotic swelling ($P_{collagen}$). Except for hyaluronan, most GAGs are proteoglycans consisting of a carbohydrate GAG attached to a core protein that interacts with the collagen matrix. Measured $P_i$ reflects a balance between matrix hydrostatic and osmotic pressures (Figure 4)

$$P_i \propto P_{collagen} - \Pi_{GAG} \quad \text{ (Eq. 12)}$$

$\Pi_i$ is primarily related to interstitial albumin concentration. The relative rates of water and albumin flux determine steady-state interstitial albumin concentration. Classically, albumin flux was assumed trivial compared with water flux with $\sigma \equiv 1$ and interstitial albumin concentration and $\Pi_i \equiv 0$. However, approximately 50 to 60% of albumin content resides in the extravascular compartment at a concentration of about 1 to 1.5 g/dl with 10 g of albumin moving from plasma to lymph per hour. Because GAGs and collagen exclude albumin from about 25 to 50% of the interstitial volume, the effective albumin concentration in the interstitium approaches 2 to 3 g/dl. $\Pi_i$ is 30 to 60% of $\Pi_{GAG}$ when directly measured and mirrors effective albumin concentration. Filtration rate dynamically regulates $\Pi_i$ with increased filtration diluting interstitial albumin and lowering $\Pi_i$, and absorption raising $\Pi_i$. $\Pi_i$ changes steeply with filtration rate as water flux outstrips albumin flux, and albumin exclusion falls producing a disproportionate decline in effective albumin concentration compared with dilution alone.

Interstitial protein gradients produce an even steeper nonlinear fall in $\Pi_i$ with filtration rate. In a two-pore model, most filtration occurs through small pores with $\sigma_{albumin} \approx 0.95$, whereas large pores transport a minute fraction of water but allow for significant albumin convection with large pore $\sigma_{albumin} \approx 0.05$. Effective albumin concentration in close proximity to small pores determines filtration as albumin infusion into the general pericapillary interstitium minimally affects filtration. Albumin gradients develop with high concentrations around large pores and low concentrations near small pores (Figure 5). Whether diffusion between pore regions is limited enough to sustain substantial protein concentration gradients is unclear. A variation of the theme proposes endothelial glyocalyx as the primary interendothelial, small pore permeability barrier with subglyocalyx $\Pi$ as the primary determinant of filtration; restricted access to the subglyocalyx space may further limit albumin diffusion to amplify interstitial concentration gradients.
collagen β1-integrin receptor interactions and/or cytoskeletal depolymerization dramatically shifts the $P_i$-volume curve rightward (Figure 6). Inflammatory states and thermal injury decrease $P_i$ as a result of reduced integrin binding of collagen and/or thermal denaturation of collagen.\textsuperscript{108–110} A fall in $P_i$ may play a critical role in the early phase of sepsis- and burn-induced edema.\textsuperscript{111,112} $P_i$ eventually rises with interstitial fluid accumulation, whereas increased $P_c$ and $L_p$ and diminished $\sigma$ maintain edema in the long term (Figure 6).\textsuperscript{107}

Dynamic interstitial forces play a critical part in regulating plasma-interstitial fluid balance in pathologic states. In hypovolemia, $P_c$ falls in vasoconstricted vascular beds leading to transient interstitial fluid absorption in a process known as transcapillary refill. Subsequent loss of interstitial volume concentrates albumin, increases $\pi$, decreases $P_i$, and thus limits transcapillary refill at approximately 75 to 80% of lost plasma volume (Figure 7).\textsuperscript{105,113,114}

Nephrotic syndrome demonstrates the interplay between serum albumin, $\pi$, capillary filtration, and $\pi$. Unlike other hypoalbuminemic states, in nephrotic syndrome the globulin $\text{MW}_{\text{av}}$ rises significantly to $\sim 215$ kD because of a preferential

![Figure 6. Dynamics of measured interstitial hydrostatic pressure $P_i$. (A) $P_i$ normally varies with interstitial volume (IFV). At low IFV, compliance is low, and pressure rises significantly. Once IFV increases 20 to 50% above euvolemia, compliance increases dramatically, and $P_i$ essentially remains near constant allowing for edema formation. (B) $P_i$ reflects a balance between $P_{\text{collagen}}$ and $\pi_{\text{GAG}}$. Release of integrin-mediated tension on collagen matrix decreases $P_{\text{collagen}}$ and right shifts the $P_i$-IFV curve, leading to increased filtration until $P_i$ rises with edema.](image-url)
Nephrotic syndrome (www.jasn.org)

urinary loss of osmotically efficient low molecular weight proteins along with albumin and an accumulation of high molecular proteins such as α1-macroglobulin, fibrinogen, haptoglobin multimers, and β lipoproteins (see supplemental material).61,62,115,116 Thus, nephrotic patients are unable to defend Πc despite a reasonable serum total protein concentration as the globulin fraction consists of large, osmotically inefficient proteins. Πc declines proportionately with falling plasma albumin as opposed to analbuminemic patients who exhibit approximately 50% of normal Πc without plasma albumin. Capillary filtration rises with falling Πc, but the subsequent fall in Πc and rise in Pc tends to normalize filtration and minimize edema (Figure 7). The difference between Πc and Πi remains constant at about 50% of Πc, or about 12 mmHg, essentially negating the tendency for low Πc to produce edema.117,118 Of course, because the floor for Πc is zero, once Πc falls below 10 to 14 mmHg (around 1.5 to 2 g/dl plasma albumin), edema is inevitable.117,119 A rapid fall in Πc, as seen in pediatric nephrotic crises with minimal change disease may kinetically outstrip the compensatory fall in Πp, leading to intravascular volume depletion and hemodynamic compromise.120–122 Whether a fall in Πc is the primary determinant of nephrotic edema is controversial, because several investigators have demonstrated autonomously enhanced sodium reabsorption in the distal nephron and diminished sodium excretion preceding hypoproteinemia.123–126 Proponents of the overfill hypothesis suggest that renal sodium retention is the primary abnormality in nephrotic syndrome, whereas others support the underfill theory wherein low Πc leads to intravascular volume depletion and secondary renal sodium retention.127,128 Increased capillary permeability measured as albumin extravasation (increased Jp, and/or decreased σ) may also contribute to intravascular volume depletion.129 As in all controversies, individual patients may demonstrate overfill, underfill, or mixed physiology. Severe or rapidly developing hypoproteinemia and clinical features of volume depletion support underfilling, whereas hypertension, renal dysfunction, and mild to moderate hypoalbuminemia (serum albumin >2 g/dl) suggest overfilling.128,130 Minimal change disease more commonly presents with underfill physiology, but whether histologic disease is an independent predictor of volume homeostasis or simply reflects more severe or rapid hypoproteinemia with maintained GFR is unclear.131

Figure 7. Transcapillary dynamics in hypovolemia and nephrotic syndrome. (A) Vasodilatation with hypovolemia decreases Pc and left shifts the Starling Πi curve with an increased y intercept. Transient interstitial fluid absorption occurs increasing Πi, and restoring a lower level of steady-state filtration. Pc also decreases leading to a small right shift in linear Starling relationship (not shown). Transcapillary refill of plasma volume occurs with absorption but may continue to occur if lymphatic flow is slow to match the shift in linear Starling relationship (not shown). Transcapillary refill of plasma volume reduces filtration to a new steady state. Πi would increase slightly producing a left shift of the Starling linear curve to further minimize a rise in filtration (not shown).

Total Body and Compartmental Tonicity

Animal cells are largely in osmotic equilibrium with their surrounding environment; thus, intracellular tonicity equals interstitial tonicity.30,132,133 Clinicians estimate plasma tonicity, which may not equal interstitial and intracellular tonicity except in the case of red blood cells (ΠRIC = Πplasma). Fortunately, in most body tissues, the difference between plasma and interstitial osmolality is minimal. A direct experimental measurement of the plasma to interstitial osmolality gradient is the difference in COP, which accounts for both protein and Donnan small ion effects96:

\[ \Pi_c - \Pi_i \approx 10–20 \text{ mmHg} \approx 0.5–1 \text{ mOsm/kg} \quad (\text{Eq. 13}) \]

The tonicity gradient between plasma and interstitium is quite small, and we can hypothesize that tonicity is equal across all body compartments:

\[ \text{Plasma Tonicity} \equiv \text{Interstitial Tonicity} \]

\[ = \text{Intracellular Tonicity} \equiv \text{Total Body Tonicity} \quad (\text{Eq. 14}) \]

In addition, if we assume the vast majority of effective body osmoles are exchangeable Na⁺ and K⁺ and their counter-ions and glucose, the following idealized relationship is derived (see supplemental material):

\[ P_{Na} = f_{PW} \cdot \frac{\text{Na}^+_{ex} + K^+_{ex}}{\text{TBW}} - (G_{Na} \cdot \Delta [\text{Glucose}]_p) - P_K \]

\[ (\text{Eq. 15}) \]

where fPW is the plasma water fraction, and GNa is a correction factor for hyperglycemia related translocational hyponatremia that has been proposed to range from 1.5 to 2.4 mEq/L per 100 mg/dl rise in plasma glucose.134–136 Studies examining the relationship between exchangeable Na⁺ and K⁺, TBW, and PNa and PK in normal and pathologic
states have found that \( P_{Na} \) may be delineated as follows (see supplemental material):

\[
P_{Na} = 1.03 \cdot f_{PW} \frac{Na^{+}_{ex} + K^{+}_{ex} - 250}{TBW} - (G_{Na} \cdot \Delta [\text{Glucose}]_{p}) - P_{K} \quad \text{(Eq. 16)}
\]

Because total exchangeable cation (\( Na^{+}_{ex} + K^{+}_{ex} \)) is on the order of 90 mEq/kg, the ideal relationship is a good approximation because 250 mEq pales in comparison and 1.03 is also quite close to 1. The 250 mEq deviation from ideality relates to a small osmotic gradient between plasma and total body osmolality, non-Na\(^+\) and K\(^+\) osmoles besides glucose, and exchangeable excess Na\(^+\) and K\(^+\) (see supplemental material). The nonideal quantity of 250 mEq in the derived \( P_{Na} \) relationship applies only to well represented pathologic states within patient cohort data (congestive heart failure, cirrhosis, and low Na\(^+\) diet). Whether this quantity is similar in other disease states such as syndrome of inappropriate anti-diuretic hormone (SIADH), volume depletion, and high Na\(^+\) diet is unknown and potentially limits the accuracy of the idealized \( P_{Na} \) approximation in these situations.\(^{137} \)

Broadly speaking, excess Na\(^+\) and K\(^+\) refer to any compartment where exchangeable Na\(^+\) and K\(^+\) concentration exceeds plasma water [Na\(^+\) + K\(^+\)]. Often, excess Na\(^+\) and K\(^+\) is used interchangeably with \textit{osmotically inactive}, although the two are not mechanistically synonymous. Some inaccurately suggest that a compartment with osmotically active Na\(^+\) and K\(^+\) in excess of plasma should accrue water until equilibrium with plasma is reached and excess cation is eliminated; thus, the persistence of a concentration gradient can only occur if excess cation is osmotically inactive. But excess cation may be osmotically active while maintaining a concentration gradient in two ways: a counteracting hydrostatic pressure balances the osmotic gradient, allowing compartment osmolality to differ from plasma osmolality or excess Na\(^+\) and K\(^+\) is counterbalanced by loss of non-Na\(^-\) and K\(^-\) osmoles maintaining plasma and total body tonicity. Of course, a portion of excess Na\(^+\) and K\(^+\) may truly be osmotically inactive (\( \varphi = 0 \)). Bone and a small fraction of intracellular cation probably represent the osmotically inactive pool. Some may argue that the distinction between excess and osmotically inactive cation is pure semantics, but a growing literature on cartilage suggests otherwise. Cartilage is hypertonic, yet inexcitable cartilage swelling is prevented by a counteracting collagen-based hydrostatic pressure.\(^{91,138} \)

Significant attention has historically focused on the dynamics of Na\(^+\) balance given its prominent role in hypertension and edematous states. When positive Na\(^+\) balance occurs, Na\(^+\) is handled in several ways: Na\(^+\) accumulates in the extracellular space with water such that plasma tonicity and Na\(^+\) concentration remain constant; Na\(^+\) is retained in excess of water with a resulting increase in plasma Na\(^+\) concentration and tonicity along with a parallel rise in total body tonicity; or Na\(^+\) is retained in excess of water with little or no change in plasma tonicity often termed \textit{excess Na\(^+\) storage} (or inaccurately as osmotically inactive Na\(^+\) storage). Broadly speaking, several mechanisms account for excess Na\(^+\) storage: negative K\(^+\) balance offsets positive Na\(^+\) balance such that total cation balance is unchanged; positive total cation balance (Na\(^+\) + K\(^+\)) with negative balance for effective osmoles other than Na\(^+\) or K\(^+\) salts such that total body effective osmoles remain constant; positive cation balance with osmotically active Na\(^+\) associating with interstitial glycosaminoglycans, thereby increasing total body tonicity relative to plasma; or positive cation balance with osmotically inactive Na\(^+\) associating with bone mineral matrix or possibly intracellular proteins (Figure 8).

Although measuring K\(^+\) balance is straightforward, defin-
ing other mechanisms for excess Na⁺ storage is difficult. Over the last half century, investigators have debated whether the movement of Na⁺ ions into or out of a storage compartment is necessary to account for positive sodium balance with high sodium diet and edematous states and negative sodium balance in volume depletion or hypotonicity. Many studies neglect K⁺ balance, which can often offset a positive Na⁺ balance. Even after accounting for K⁺ balance, most short term metabolic balance studies (3 to 5 days) and long term (1 to 3 months) radioisotopic studies generally find reasonable correlation between cation and water balance and plasma tonicity, whereas intermediate balance studies (7 to 14 days) often suggest Na⁺ storage. Whether these differences reflect biologic phenomena or technical differences remains unclear.

Excess Na⁺ storage occurs with high sodium diet (>300 mEq/d). Some excess Na⁺ exchanges with intracellular K⁺ primarily from skeletal and vascular smooth muscle; thus, total cation balance in this case is unchanged. Excess Na⁺ storage in skin exceeds negative K⁺ balance and has recently garnered attention, although the hypothesis dates back over 30 years in the Russian literature. Animal studies demonstrate increased Na⁺ concentration in skin (20 to 40 mEq/L tissue water), which translates to about 1 to 2 mEq/kg excess Na⁺ storage, assuming that skin water is at most 5% of body weight. Excess Na⁺ may accumulate intracellularly in exchange for non-K⁺ osmotically inactive storage with interstitial glycosaminoglycans (Figure 8). Radiotracer Na⁺ dynamics in isolated skin from animals on high sodium diets suggest an increase in the rapidly exchanging extracellular Na⁺ pool rather than the more slowly exchanging intracellular Na⁺ pool. Within the rapidly exchanging pool, a compartment outside the inulin space accounts for the majority of the increased Na⁺ content, suggesting a sterically inaccessible site such as interstitial glycosaminoglycans. Indeed, negatively charged, sulfated glycosaminoglycan content rises in skin with dietary Na⁺ loading. Na⁺ loading transiently increases interstitial fluid and possibly Na⁺ concentration, which are known stimulators of interstitial cell matrix production, particularly sulfated GAGs.

Na⁺ associated with negatively charged purified glycosaminoglycans in cartilage exhibits an osmotic coefficient similar to normal saline; thus, skin GAG-associated Na⁺ is most likely osmotically active. Assuming that 20 to 40 mEq/L Na⁺ is stored in association with skin interstitial GAGs, ΠGAG would rise about 100 to 200 mmHg or approximately 5 to 10 mOsm/kg to achieve the required negative charge density (see supplemental material). Typical transcapillary Starling forces pale in comparison with 100 to 200 mmHg. Dermal swelling pressures can reach up to 100 to 150 mmHg in situations where Pcollagen is reduced, suggesting that ΠGAG is normally quite high but counteracted by Pcollagen. Thus, ΠGAG can rise significantly but requires similar increases in Pcollagen to prevent high filtration rates and interstitial edema caused by low P↓ (P↓ > Pcollagen − ΠGAG). Hydrostatic pressure (Pcollagen) counterevolves osmotic pressure (ΠGAG) to eliminate water movement and maintain a small tonicity gradient between dermal interstitium and plasma. Dermal fibroblasts like their chondrocyte counterparts probably accommodate interstitial hypertonicity with osmolyte accumulation to maintain cell volume.

When the mechanism of excess Na⁺ storage across tissues is broadly surveyed, a hypothetical framework comes into view. Relative or cell mass relative to interstitial space provide a large osmole depot for transcellular exchange. Conversely, relatively acellular connective tissues have minimal intracellular osmoles at their disposal and alternatively depend on osmotically active storage with interstitial glycosaminoglycans or osmotically inactive storage with mineral matrix.

The Na⁺ counter-anion may also modify Na⁺ storage. When subjects consume large amounts of Na⁺ either as Cl⁻ or bicarbonate (or equivalents such as citrate or ascorbate) salts, hypertension and plasma volume expansion ensues only with NaCl intake despite equivalent positive sodium balance and weight gain with sodium bicarbonate. Furthermore, NaCl consumption results in hypercalciuria, whereas sodium bicarbonate does not change urinary calcium excretion. Taken together, water distribution tends to be extravascular and does not affect calcium homeostasis when the Na⁺ counter-anion is bicarbonate. Na⁺ with a base equivalent may possess a larger pool of intracellular and bone storage mechanisms. Intracellular proteins can simply titrate bicarbonate with protons with the resulting protein anionic side chain acting as a counter-ion for excess Na⁺ in a potentially osmotically inactive form. For NaCl to store Na⁺ in association with intracellular proteins, Na⁺ would have to displace protein side chain H⁺ or exchange with predominantly protein bound Ca²⁺. The former is unlikely because cells function poorly with intracellular acidosis, whereas the latter necessitates Ca²⁺ excretion. The low solubility of calcium bicarbonate probably precludes intracellular Na⁺/Ca²⁺ exchange with sodium bicarbonate but promotes bone surface crystal integration of sodium bicarbonate in toto. Conversely, NaCl requires Na⁺/Ca²⁺ exchange at the bone matrix interface again leading to hypercalciuria. Thus, negative Ca²⁺ balance potentially limits Na⁺ storage in the setting of high NaCl intake, but not with bicarbonate salts. Although purely speculative, these hypotheses provide fertile ground for future investigation.

Alterations in intracellular, skin, and bone Na⁺ storage may critically regulate blood volume homeostasis and participate in the pathogenesis of salt-sensitive hypertension. These storage mechanisms may buffer the blood volume against transient or sustained sodium loads. Animals and patients with reduced Na⁺ storage capacity are prone to blood volume expansion and hypertension. Alternatively, these storage mechanisms activate deleterious neurohormonal and/or inflammatory signaling pathways. Although this work points to an exciting paradigm shift in blood volume regulation, whether
these mechanisms contribute to pathology broadly or in a narrow subset of patients remains unknown. Because United States dietary Na⁺ intake is 150 to 200 mEq/d ± 100 mEq/d (2 SD)¹⁷⁹–¹⁸¹ and storage mechanisms regulating Na⁺ homeostasis require dietary intakes exceeding 300 mEq/d,¹⁴⁰,¹⁵⁰ only about 5% of essential hypertension in American patients may involve alterations in Na⁺ storage.

Conclusions
Understanding body fluid dynamics is critical to the practice of medicine. Phenomenal work accomplished during the last century has lulled us into relying on aging textbook dogma or believing there is little left to discover. However, re-examination of foundational literature suggests some teachings stray from original data. The division of TBW into ICF and ECF is frequently taught as an arbitrary distribution rather than a product of cell volume homeostasis and the relative partitioning of body fat, protein, Na⁺, and K⁺. New investigations also suggest novel paradigms involving the dynamic nature of the interstitium that critically regulate ECF homeostasis. Although diet and the kidneys arbitrate blood volume homeostasis in the long run, the interstitium plays a larger role in short term blood and interstitial volume adjustments. Short term Na⁺ storage and interstitial volume homeostasis may be relevant to transient or nonequilibrium phenomena such as BP dipping, flash pulmonary edema, rapid blood loss, burns, and sepsis, to name a few. Future investigation will hopefully unify the molecular and structural biology of interstitial cell-matrix interactions with classic Starling physiology to identify new therapeutic targets for hemodynamic derangements.

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DISCLOSURES
None.

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Supplemental Materials

1. Index of Mathematical Symbols
2. Anthropomorphic Equations for TBW
3. Qualitative Calculation of Hypothetical Urea Gradients
4. Average Plasma Protein Molecular Weight in Low Albumin States
5. Plasma Tonicity to Total Body Tonicity Relationship
6. Estimation of Fixed Charge Density and $\Pi_{GAG}$ for Skin Na$^+$ Storage

Supplemental Table 1. Plasma Protein MW<sub>av</sub> Calculations in Various Low Albumin States
Supplemental Table 2. Linear Regression Analysis of Plasma and Body Tonicity Relationships
Supplemental Table 3. Exchangeable Excess Cation Balance

1. Index of Mathematical Symbols

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<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>$M_u$</td>
<td>Urea mass transfer rate</td>
</tr>
<tr>
<td>$G_u$ or $R_u$</td>
<td>Urea generation or loss rate</td>
</tr>
<tr>
<td>$\Delta[C]_u$</td>
<td>Hypothetical urea gradient (transcellular or transcapillary)</td>
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<tr>
<td>$PS_{\text{urea}}$</td>
<td>Permeability * Surface Area product or urea diffusion capacity; calculated for entire surface area of all cell membranes ($PS_{\text{urea-cm}}$) or cerebral capillaries ($PS_{\text{urea-brain}}$)</td>
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<tr>
<td>$\Pi$</td>
<td>Osmotic Pressure in mm Hg</td>
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<tr>
<td>MW&lt;sub&gt;av&lt;/sub&gt;</td>
<td>Average molecular weight of a protein or mixture of proteins determined using limiting slope of the osmotic pressure-protein concentration relationship</td>
</tr>
<tr>
<td>$\phi$</td>
<td>True osmotic coefficient of a solute</td>
</tr>
<tr>
<td>$d\Pi / dc$</td>
<td>Slope of the osmotic pressure-protein concentration relationship as protein concentration approaches zero (“limiting slope”)</td>
</tr>
<tr>
<td>$z_{\text{eff}}$</td>
<td>Effective valence of a salt; for example $z_{\text{eff}}$ for NaCl equals 2. Note that $z_{\text{eff}}$ for higher order salts is not as easily predicted with significant non-linear behavior.</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Effective osmotic coefficient which takes into account counter-ion since it multiplies true osmotic coefficient by effective valence.</td>
</tr>
<tr>
<td>$\phi_{\text{Na}^+K}$</td>
<td>Effective osmotic coefficient of plasma Na$^+$ and K$^+$</td>
</tr>
<tr>
<td>$\phi_{\text{bNa}^+K}$</td>
<td>Effective osmotic coefficient of total body Na$^+$ and K$^+$</td>
</tr>
<tr>
<td>$\phi_{\text{i}}$</td>
<td>Effective osmotic coefficient of the “i”&lt;sup&gt;th&lt;/sup&gt; non-Na$^+$, non-K$^+$ plasma solute</td>
</tr>
<tr>
<td>$\phi_{\text{bi}}$</td>
<td>Effective osmotic coefficient of the “i”&lt;sup&gt;th&lt;/sup&gt; non-Na$^+$, non-K$^+$ total body solute</td>
</tr>
<tr>
<td>$V_{\text{Di}}$</td>
<td>Volume of distribution as a fraction of TBW of the “i”&lt;sup&gt;th&lt;/sup&gt; non-Na$^+$, non-K$^+$ total body solute</td>
</tr>
<tr>
<td>$\phi_{\text{heNa}^+K}$</td>
<td>Effective osmotic coefficient for non-exchangeable total body Na$^+$ and K$^+$; essentially equals zero.</td>
</tr>
<tr>
<td>$\phi_{\text{eNa}^+K}$</td>
<td>Effective osmotic coefficient for exchangeable total body Na$^+$ and K$^+$.</td>
</tr>
<tr>
<td>$m_{\text{osm}}, b_{\text{osm}}$</td>
<td>Slope &amp; y-intercept of the general relationship between plasma and total body osmolality</td>
</tr>
<tr>
<td>Na\textsuperscript{+} \textsubscript{ex} + K\textsuperscript{+} \textsubscript{ex}</td>
<td>Radioisotope exchangeable Na\textsuperscript{+} plus exchangeable K\textsuperscript{+}. Typical exchange times are 24 hours for Na\textsuperscript{+} and 40-48 hours for K\textsuperscript{+}.</td>
</tr>
<tr>
<td>Na\textsuperscript{+} \textsubscript{eA} + K\textsuperscript{+} \textsubscript{eA}</td>
<td>Excess (or “residual”) Na\textsuperscript{+} and K\textsuperscript{+} which is osmotically active; the latter is defined as an osmotic coefficient approaching $\phi_{Na+K}$.</td>
</tr>
<tr>
<td>Na\textsuperscript{+} \textsubscript{el} + K\textsuperscript{+} \textsubscript{el}</td>
<td>Excess (or “residual”) Na\textsuperscript{+} and K\textsuperscript{+} which is osmotically inactive with an osmotic coefficient of near zero.</td>
</tr>
<tr>
<td>f\textsubscript{PW}</td>
<td>Plasma water fraction – usually about 0.93 but may fall with hyperproteinemia or hyperlipidemia. Allows one to convert molar concentrations to molal concentrations: Molal concentration * f\textsubscript{PW} = Molar Concentration</td>
</tr>
<tr>
<td>[S]\textsubscript{i}</td>
<td>Plasma molar concentration of the “i\textsuperscript{th}” non-Na\textsuperscript{+}, non-K\textsuperscript{+} solute.</td>
</tr>
<tr>
<td>TB [s]\textsubscript{i}</td>
<td>Total body molal concentration of the “i\textsuperscript{th}” non-Na\textsuperscript{+}, non-K\textsuperscript{+} solute.</td>
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<tr>
<td>G\textsubscript{Na}</td>
<td>Corrects plasma [Na\textsuperscript{+}] for hyperglycemia related translocational hyponatremia. Proposed to range from 1.5 – 2.4 mEq/L fall in plasma [Na\textsuperscript{+}] per 100 mg/dL rise in plasma glucose.</td>
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<td>[Na\textsuperscript{+}]\textsubscript{GAG}</td>
<td>Na\textsuperscript{+} and Cl\textsuperscript{−} concentrations in the interstitial GAG phase of the three phase model.</td>
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<td>[Cl\textsuperscript{−}]\textsubscript{GAG}</td>
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<tr>
<td>[Na\textsuperscript{+}]\textsubscript{lf}</td>
<td>Na\textsuperscript{+} and Cl\textsuperscript{−} concentrations in the interstitial “free fluid” phase of the three phase model.</td>
</tr>
<tr>
<td>[Cl\textsuperscript{−}]\textsubscript{lf}</td>
<td></td>
</tr>
<tr>
<td>FCD</td>
<td>Fixed charge density – concentration of interstitial GAG negative charge</td>
</tr>
</tbody>
</table>

2. Anthropomorphic Equations for TBW

Watson et al.:\textsuperscript{1}
Males: $0.1074 \times \text{Height} + 0.3362 \times \text{Weight} - 0.09516 \times \text{Age} + 2.447$
Females: $0.1069 \times \text{Height} + 0.2466 \times \text{Weight} - 2.097$

Chumlea et al.:\textsuperscript{2}
Caucasian Males: $-0.62 \times \text{BMI} + 0.5 \times \text{Weight} - 0.03 \times \text{Age} - 23.04$
Caucasian Females: $0.18 \times \text{Height} + 0.2 \times \text{Weight} - 0.01 \times \text{Age} - 10.5$
African American Males: $0.25 \times \text{Height} + 0.34 \times \text{Weight} - 0.09 \times \text{Age} - 18.37$
African American Females: $0.24 \times \text{Height} + 0.22 \times \text{Weight} - 0.05 \times \text{Age} - 16.71$

Ellis:\textsuperscript{3}
Males: $0.284 \times \text{Height} + 0.25 \times \text{Weight} - 0.092 \times \text{Age} - 21.9$
Females: $0.273 \times \text{Height} + 0.17 \times \text{Weight} - 0.045 \times \text{Age} - 21.9$

Units: TBW in liters, Height in cm, Weight in kg, Age in years, and BMI in kg/m\textsuperscript{2}
3. Qualitative Calculation of Hypothetical Urea Gradients

These calculations are designed only to qualitatively illustrate underlying physiologic principles. We assume no renal excretion or extrarenal metabolism of urea as a simplification. To calculate a potential urea concentration gradient (Δ[C]u), we equate urea generation rate (G_u) to total urea mass transfer rate (M_u) and solve for Δ[C]u:

\[ M_u = \text{PS}_{\text{urea-cm}} \times \Delta[C]_u = G_u \]

\[ \Delta[C]_u = G_u + \text{PS}_{\text{urea-cm}} \]

where \( \text{PS}_{\text{urea-cm}} \) equals the cell membrane diffusive capacity for urea. To calculate \( \text{PS}_{\text{urea-cm}} \) area, we use the average urea permeability coefficient for artificial lipid bilayers (\( P_D \sim 3 \times 10^{-6} \text{ cm/s} \)) and envision an average human spherical cell with a radius of 10 \( \mu \text{m} \) and assume that there are 10\(^{13} \) cells in an average adult human.\(^{4-6} \)

\[
\text{PS}_{\text{urea-cm}} = 3 \times 10^{-6} \text{ cm/s} \times 4 \times \pi \times (0.001 \text{ cm})^2 \times 10^{13} = 22.6 \text{ L/min}
\]

Based on literature estimates for \( G_u \) in normal subjects, \( \Delta[C]_u \) can be calculated as follows:\(^7,8 \)

\[
G_u = 0.5 \text{ mmol/min (~ 40 g urea/day)}
\]

\[
\Delta[C]_u = 0.46 \text{ mmol/min} + 22.6 \text{ L/min}
\]

\[
\Delta[C]_u \approx 0.025 \text{ mM}
\]

In essence, the very large cell surface area (~ 1.25 \( \times 10^8 \text{ cm}^2 \) or 12,500 m\(^2 \)) minimizes transcellular urea gradients despite low permeability (\( P_D \)). A similar approach can be taken to calculate a cerebral transcapillary urea gradient during high efficiency hemodialysis as BUN falls at a rate of 50 mg/dL/hour which equals a blood [urea] rate (ΔBlood [urea]) of 17.8 mM/h. A total urea mass removal rate, \( R_u \), from cerebral blood is first calculated:

\[
R_u = \text{CBV} \times \Delta\text{Blood [urea]}
\]

\[
\text{CBV} = 0.063 \text{L}
\]

\[
R_u = 0.019 \text{ mmol/min}
\]

where CBV is cerebral blood volume.\(^9 \) To delineate transcapillary urea mass transfer rate, total brain urea
capillary diffusion capacity ($PS_{\text{urea-brain}}$) is used to obtain a potential transcapillary urea concentration gradient:\(^\text{10}\)

$$PS_{\text{urea-brain}} \approx 75 \text{ mL/min}$$

$$\Delta[C]_u = R_u + PS_{\text{urea-brain}}$$

$$\Delta[C]_u = 0.019 \text{ mmol/min} + 0.08 \text{ L/min} \approx 0.25 \text{ mM}$$

### 4. Average Plasma Protein Molecular Weight in Low Albumin States

Average protein molecular weight ($MW_{av}$) may be calculated from the relationship between protein osmotic pressure and concentration:

$$\Pi (37^\circ\text{C}; \text{ mm Hg}) = 19.34 \times \phi \times [c]$$

$$\Pi (37^\circ\text{C}; \text{ mm Hg}) = (19.34 \times \phi \times 10 \times c) + (MW_{av} \times f_W)$$

where $\phi$ is the osmotic coefficient (see above), $[c]$ is molal protein concentration, $f_W$ is the fraction of water in the solution, and $c$ is concentration in g/dL. As protein concentration approaches zero, $\phi$ and $f_W$ approach 1, and the limiting slope ($d\Pi / dc$) of the relationship provides $MW_{av}$:

$$MW_{av} (\text{in kD}) = 193.4 \div \frac{d\Pi}{dc} (c = 0)$$

As a first approximation, $MW_{av}$ provides an estimate of osmotic efficiency with lower molecular weight proteins generating greater osmotic pressure per g/dL of protein. Higher order osmotic virial coefficients (see above) may alter the $MW_{av}$ osmotic efficiency relationship particularly at higher protein concentrations, but the $MW_{av}$ concept is qualitatively useful to illustrate determinants of plasma COP. More importantly, $MW_{av}$ of protein mixtures ($MW_{av-mix}$) may be calculated as follows:\(^\text{11}\)

$$\frac{1}{MW_{av-mix}} = \sum \frac{f_i}{MW_{av-i}}$$

where $f_i$ is the fraction of the $i^{th}$ protein component and $MW_{av-i}$ the average MW of that component. Using this relationship and literature estimates, plasma protein average molecular weight may be calculated in various
Bhave and Neilson

5. Plasma Tonicity to Total Body Tonicity Relationship

The osmotic coefficient $\varphi$ relates solute concentration to osmotic pressure $\Pi$ for non-dilute solutions:

$$\Pi (37^\circ C; \text{mm Hg}) = 19.34 \times \varphi \times z \times [c]$$

where $[c]$ represents molal solute concentration and $z$ is valence for electrolyte solutes. While $\varphi$ is often denoted as a constant, it actually is a polynomial function of molal solute concentration:

$$\varphi = 1 + \varphi_1 \times [c] + \varphi_2 \times [c]^2 + \varphi_3 \times [c]^3 + \ldots$$

where $\varphi_1$, $\varphi_2$, and $\varphi_3$ are osmotic virial coefficients of any value including less than zero. As $[c]$ approaches zero (increasingly dilute solution), the higher order terms become inconsequential and $\varphi$ first approaches $1 + \varphi_1$ and then approaches the ideal value of 1. Most salts at physiologic concentrations are aptly described by $1 + \varphi_1$; thus osmotic pressure may be expressed as a function of a constant osmotic coefficient. However, body fluids are mixtures of solutes including proteins with complex osmotic behavior. A composite osmotic coefficient $\varphi_c$ may be defined for a solution with $n$ solutes:

$$\Pi (37^\circ C; \text{mm Hg}) = 19.34 \times \varphi_c \times \sum z_i \times [c]_i$$

Analogous to single solute solutions, $\varphi_c$ is not constant but a complex function of each solute concentration with interaction terms between solutes. For example, $\varphi_c$ for plasma will vary with plasma $[Na^+]$, glucose, and other solute concentrations. At the limited physiologically relevant solute concentrations, we assume minimal interaction between solutes and express the osmotic pressure of the mixed solution as the sum of its component osmotic pressures:

$$\Pi (37^\circ C; \text{mm Hg}) = 19.34 \times \sum z_i \times \varphi_i \times [c]_i$$

$$\varphi_c = (\sum z_i \times \varphi_i \times [c]_i) + (\sum z_i \times [c]_i)$$
Experimentally, individual osmotic coefficients may be obtained by measuring the change in osmolality with addition of a given solute to the body fluid, while other solute concentrations are held relatively constant.\textsuperscript{24, 25}

Since body cation salts have many counterions (chloride, bicarbonate, phosphate, albumin, intracellular proteins, etc.), an effective valence ($z_{\text{eff}}$) and osmotic coefficient ($\phi$) will be used throughout:

\[
\phi_{\text{cation}} = \phi_{\text{cation}} \cdot z_{\text{eff}}
\]

With this construct, expressions for plasma and total body osmolality may be derived:

Plasma $[c] = $ Plasma $[C] \div f_{PW}$

\[
P_{\text{osm}} = \phi_{p\text{Na}^+K^+} \cdot P_{\text{Na}^+K^+} \div f_{PW} + \sum \phi_{pi} \cdot \text{Plasma } [S] / f_{PW}
\]

Total Body $[c] = $ TB $[C] \div TBW$

\[
\phi_{tb} = \phi_{tb} \cdot z_{\text{eff-tb}}
\]

\[
TB_{\text{osm}} = \phi_{tb\text{Na}^+K^+} \cdot \frac{\text{TB Na}^+ + \text{TB K}^+}{TBW} + \sum \phi_{tbi} \cdot \frac{\text{TB S}_i}{TBW}
\]

where $[c]$ and $[C]$ represent molal and molar concentrations respectively, $f_{PW}$ is the plasma water fraction (normally $\sim 0.93$), $\phi_{p\text{Na}^+K^+}$ is the effective osmotic coefficient for plasma Na\textsuperscript{+} and K\textsuperscript{+} salts, and $\phi_{tb\text{Na}^+K^+}$ is the effective osmotic coefficient for total body Na\textsuperscript{+} and K\textsuperscript{+} salts. Note that $\phi_{p\text{Na}^+K^+}$ and $\phi_{tb\text{Na}^+K^+}$ both account for Na\textsuperscript{+} and K\textsuperscript{+} as well as their accompanying anions with the inclusion of effective valence ($z_{\text{eff}}$). $S$ represents “n” non-Na\textsuperscript{+} and K\textsuperscript{+} cation salts and uncharged substances, which are multiplied by their respective osmotic coefficients and summed ($\sum$). Plasma non-Na\textsuperscript{+} and K\textsuperscript{+} solutes may be related to total body solute content (TB $S$) using the solute volume of distribution ($V_D$) expressed as a fraction of TBW:

\[
\frac{f_{PW}}{V_{Di}} \cdot \text{Plasma } [S]_i = \frac{\text{TB} [s]_i}{TBW}
\]

Total body Na\textsuperscript{+} and K\textsuperscript{+} is divided into non-exchangeable (Na\textsuperscript{+}_{ne} + K\textsuperscript{+}_{ne}) and exchangeable (Na\textsuperscript{+}_{ex} + K\textsuperscript{+}_{ex}) fractions:
TB \( \text{Na}^+ + \text{TB} \, \text{K}^+ = (\text{Na}^+_{\text{ne}} + \text{K}^+_{\text{ne}}) + (\text{Na}^+_{\text{ex}} + \text{K}^+_{\text{ex}}) \)

\[
\frac{\Phi_{\text{tbNa}+K} \cdot \text{TB} \, \text{Na}^+ + \text{TB} \, \text{K}^+}{\text{TBW}} = \frac{\Phi_{\text{heNa}+K} \cdot \text{Na}^+_{\text{ne}} + \text{K}^+_{\text{ne}}}{\text{TBW}} + \frac{\Phi_{\text{eNa}+K} \cdot \text{Na}^+_{\text{ex}} + \text{K}^+_{\text{ex}}}{\text{TBW}}
\]

Non-exchangeable \( \text{Na}^+ \) and \( \text{K}^+ \) do not affect body water distribution; thus \( \Phi_{\text{heNa}+K} = 0 \) and total body \( \text{Na}^+ \) and \( \text{K}^+ \) osmolality equals exchangeable \( \text{Na}^+ \) and \( \text{K}^+ \) osmolality.

Plasma and total body osmolality may differ. We relate \( P_{\text{osm}} \) and \( \text{TB}_{\text{osm}} \) as a linear relationship over the limited physiologic range of osmolality:

\[ P_{\text{osm}} = m_{\text{osm}} \cdot \text{TB}_{\text{osm}} + b_{\text{osm}} \]

Using the relationship between \( P_{\text{osm}} \) and \( \text{TB}_{\text{osm}} \), we can derive a relationship for \( P_{\text{Na}} \):

\[
P_{\text{Na}} = \frac{f_{\text{PW}} \cdot m_{\text{osm}}}{\Phi_{\text{pNa}+K}} \left( \frac{\Phi_{\text{eNa}+K} \cdot \text{Na}^+_{\text{ex}} + \text{K}^+_{\text{ex}}}{\text{TBW}} \right) + \frac{f_{\text{PW}} \cdot b_{\text{osm}}}{\Phi_{\text{pNa}+K}} + \sum \text{TB} \left[ s \right] \cdot (\Phi_{\text{lb}i} - \Phi_{\text{pb}} / V_{\text{Di}}) - P_{\text{K}}
\]

The summation term of non-\( \text{Na}^+ \) and \( \text{K}^+ \) solutes allows one to delineate effective and ineffective osmoles and their effect on \( P_{\text{Na}} \). Assuming minimal differences in plasma and total body osmotic coefficients (\( \Phi_{\text{lb}i} \approx \Phi_{\text{pb}} \)), if the solute distributes throughout TBW with \( V_{\text{Di}} \equiv 1 \), then the solute concentration is equal in all body compartments and changes in body solute content do not affect \( P_{\text{Na}} \); the solute behaves as an ineffective osmole.

Urea and ethanol permeate into all body water compartments, distribute into TBW, and do not affect \( P_{\text{Na}} \).

Plasma solutes residing in the ECF such as glucose or mannitol exhibit \( V_{\text{D}} < 1 \) and lower \( P_{\text{Na}} \) since \( \Phi_{\text{p} \text{Glucose}} - (\Phi_{\text{p} \text{Glucose}} / V_{\text{DGlucose}}) \) is less than zero. Conversely, solutes with higher tissue concentrations compared to plasma have a \( V_{\text{D}} > 1 \) and raise \( P_{\text{Na}} \) as \( \Phi_{\text{h} \text{Si}} - (\Phi_{\text{p} \text{Si}} / V_{\text{Dsi}}) > 0 \). Intuitively, intracellular solutes draw water into cells raising \( P_{\text{Na}} \), while extracellular solutes tend to pull water into the ECF lowering \( P_{\text{Na}} \).

To incorporate exchangeable, excess \( \text{Na}^+ \) and \( \text{K}^+ \) into the \( P_{\text{Na}} \) relationship, exchangeable \( \text{Na}^+ \) and \( \text{K}^+ \)
may be modeled as two components; one iso-osmolar to plasma Na\(^+\) and K\(^+\) distributed across TBW and another representing excess Na\(^+\) and K\(^+\) distributed across TBW and divided into osmotically active and inactive components. The osmotically active component (Na\(^+_eA\) + K\(^+_eA\)) is assumed to have an osmotic coefficient equal to or near \(\phi_{pNa+K}\), while the inactive component (Na\(^+_eI\) + K\(^+_eI\)) is assumed to have a coefficient equal to zero (or \(<< \phi_{pNa+K}\)):

\[
Na^+_e + K^+_e = (TBW \times P_{Na+K} / f_{PW}) + (Na^+_eA + K^+_eA) + (Na^+_eI + K^+_eI)
\]

\[
\frac{Na^+_e + K^+_e}{TBW} = \frac{q_{Na+K} \times P_{Na+K}}{TBW} + \frac{Na^+_eA + K^+_eA}{TBW} - \frac{q_{Na+K}}{TBW}
\]

A complete relationship for \(P_{Na}\) may be derived incorporating osmotically inactive Na\(^+\) and K\(^+\): \(^{26}\)

\[
P_{Na} = \frac{f_{PW} \times m_{osm}}{q_{Na+K}} \left( \frac{q_{Na+K} \times Na^+_e + K^+_e}{TBW} + \sum TB [s] \times (q_{libi} - q_{p/V_Di}) + \frac{P_{K}}{q_{Na+K}} \right)
\]

\[
P_{Na} = f_{PW} \times m_{osm} \left( \frac{Na^+_e + K^+_e}{TBW} - \frac{Na^+_e + K^+_{el}}{TBW} \right) \times \left( \frac{f_{PW} \times m_{osm}}{q_{Na+K}} \sum TB [s] \times (q_{libi} - q_{p/V_Di}) + \frac{b_{[osm]}}{m_{osm}} \right) - P_{K}
\]

We can also relate osmotically active excess cation to changes in \(P_{Na+K}\), non-Na\(^+\) and K\(^+\) osmoles, and \(b_{[osm]}\):

\[
\frac{Na^+_eA + K^+_eA}{TBW} = \frac{q_{Na+K} \times (1 - m_{osm})}{f_{PW} \times m_{osm}} \times \frac{1}{q_{Na+K}} \sum TB [s] \times (q_{libi} - q_{p/V_Di}) + \frac{b_{[osm]}}{m_{osm}}
\]
The P_{Na} relationship appears complex, but if P_{Na} is plotted against Na^{+}_{ex} + K^{+}_{ex}/TBW, a linear relationship should result with a slope of f_{PW} * m_{osm} and a y-intercept determined by a balance between osmotically inactive excess Na^{+} and K^{+} against non-Na^{+} and K^{+} osmoles and a potential difference in plasma to total body osmolality. As expected changes in the osmotically inactive cation do not change P_{Na} since total exchangeable Na^{+} + K^{+} and inactive cation both change equally and cancel each other. Changes in osmotically active excess Na^{+} and K^{+} will either change the plasma to total body tonicity relationship (m_{osm} and/or b_{[osm]}) or non-Na^{+} and K^{+} solutes. P_{Na+K} changes minimally, since m_{osm} is close to 1 blunting P_{Na+K} impact on osmotically active excess cation.

In pioneering work, Edelman delineated a significant linear relationship between molal P_{Na} as the dependent variable and (Na^{+}_{e} + K^{+}_{e}) ÷ TBW as the independent variable with a slope of 1.11 and a y-intercept of -25.6. Other investigators note a weaker relationship between P_{Na} and (Na^{+}_{e} + K^{+}_{e}) ÷ TBW. Conventional linear regression between P_{Na} and (Na^{+}_{e} + K^{+}_{e}) ÷ TBW must be approached carefully as imprecise or inaccurate estimates may result due to non-normal distribution, narrow data range, and measurement error in the independent variable. A reasonable analogy has been drawn to issues with linear regression to compare analytic methods in clinical laboratories, such as validation of a routine technique against a gold standard. Since both methods attempt to measure the same quantity, significant correlation is expected, but establishing whether such correlation translates into quantitative prediction of constant (y-intercept) and proportional (slope) differences between methods is often difficult. Since both independent and dependent variables are measured, measurement error in the independent variable is always present. Error in the independent variable tends to lower the slope and raise the y-intercept often termed dilution or attenuation bias. Linear regression techniques adapted for independent variable measurement error may mitigate this issue. Indeed, Edelman’s study recognized this issue and most likely used Deming linear regression to adjust for dilution bias. Some laboratory measurements such as plasma K^{+} have inherently narrow data ranges which require large sample sizes to accurately determine method bias. Similarly, P_{Na} may range from 110 to 170 mEq/L or by a factor of
~1.5 requiring a sample size minimally greater than 100 and usually greater than 500-1000 to accurately determine equivalency.\textsuperscript{34} While statistical variants of linear regression can reduce sample size requirements for narrow data sets, the resulting parameters often have broad confidence intervals. The ideal solution is a large enough sample size resulting in convergence of estimates from simple and complex linear regression algorithms.\textsuperscript{35} Data transformation may expand the data range and reduce sample size requirements. In follow-up to the Edelman study, Boling and Olesen noted that the \((Na^+ + K^+)\) versus \(([Na^+ + K^+]_p \times TBW)\) total body cation relationship improved linear regression probably due to a wider data set (~2500 – 10,000 mEq; factor of 4).\textsuperscript{28,30} Finally, \(P_{Na}\) data sets often exhibit positive skew as hyponatremia tends to be more commonly studied than hypernatremia; non-parametric linear regression such as Passing-Bablok may be useful in this circumstance.\textsuperscript{36,37}

Linear regression variations of the Edelman and pooled (Edelman, Boling, and Olesen) data sets are presented in Supplemental Table 2.\textsuperscript{27,28,30} When the \(P_{Na+K}\) relationship is used, the simple linear regression estimates diverge significantly from the Deming and Passing-Bablok estimates suggesting inadequate sample size for the narrow data range. However, the slope and y-intercept estimates using the total body cation relationship are quite similar regardless of regression technique and reveals a slope of ~0.97 with molal \([Na^+ + K^+]_p\) and a y-intercept of about ~250 mEq. The \(P_{Na}\) relationship can now be simplified to:

\[
P_{Na} = 1.03 \times f_{PW} \frac{Na^+_c + K^+_c - 250}{TBW} - P_K
\]

The relationship delineates a \(m_{osm}\) of 1.03 and the net balance of osmotically inactive \(Na^+\) and \(K^+\) cation against non-\(Na^+\) and \(K^+\) solutes and plasma to total body osmolality difference (\(b_{osm}\)) to be about 250 mEq. \(m_{osm}\) is slightly greater than 1 suggesting that the ratio of exchangeable cation to TBW underestimates \(P_{Na}\), while the negative y-intercept suggests the opposite. Only the interstitial and transcellular compartments are known to have cation concentrations below \(P_{Na+K}\); thus these components must underlie the deviation of \(m_{osm}\) from the ideal of 1. All other compartments with cation concentrations higher than plasma must lead to the
overestimation of $P_{Na}$ by the $(Na^{+}_{ex} + K^{+}_{ex}) \div TBW$ and contribute to the y-intercept. The non-$Na^{+}$ and $K^{+}$ plasma predominant osmoles (summation term negative) or a plasma and total body osmolality gradient (b[osm] negative with total body osmolality greater than plasma) primarily mirror osmotically active excess cation. In supplemental Table 3, we present compartments, estimate excess cation in these compartments, and delineate their effects on $m_{osm}$ and the y-intercept of the $P_{Na}$ relationship.

Translocational hyponatremia related to hyperglycemia (or other extracellular osmoles) is a common clinical situation where hyponatremia is not related to changes in exchangeable $Na^{+}$, $K^{+}$, or TBW, yet the $P_{Na}$ relationship doesn’t appear to account for these situations. Since the classic body composition studies examined euglycemic or mildly hyperglycemic patients, the narrow range of plasma glucose values prevented the investigators from delineating a relationship between $P_{Na}$ and glucose.\(^{27}\) To account for hyperglycemia, a revised relationship may be obtained:

$$P_{Na} = 1.03 \times f_{PW} \times \frac{\left(\frac{Na^{+}_{ex} + K^{+}_{ex} - 250}{TBW}\right)}{(G_{Na} \times \Delta [Glucose]_p) - P_{K}}$$

$$G_{Na} = \frac{1.03 \times f_{PW} \times \left((\phi_{tbGlucose} \times V_{DGluose}) - \phi_{pGlucose}\right)}{\phi_{pNa+K}}$$

where $G_{Na}$ is a correction factor for hyperglycemia related translocational hyponatremia which has been proposed to range from 1.5 – 2.4 mEq/L per 100 mg/dL rise in plasma glucose (\(\Delta [Glucose]_p\)).\(^{38-40}\)

Excess $Na^{+}$ and $K^{+}$ or non-$Na^{+}$ and $K^{+}$ solutes may change in pathologic states leading primarily to changes in the $P_{Na}$ relationship y-intercept. The linear regression analysis yields a relatively fixed value for the y-intercept of 250 mEq (+ TBW). The broad range of conditions in the study cohorts used for regression would suggest that excess $Na^{+}$ and $K^{+}$ is relatively fixed.\(^{41}\) But edematous states seem overrepresented, while conditions such as SIADH, high sodium diet, volume depletion, and hyperglycemia are relatively absent\(^{27, 28, 30}\). Thus, the y-intercept in the $P_{Na}$ relationship probably holds true for many disease states, but may not hold true for underrepresented states. For example, the y-intercept requires modification to account for hyperglycemia
due to shortcomings in the derivation cohorts.

At the bedside, clinicians use a shortcut for the P_{Na} relationship:\(^{42,43}\)

\[
P_{Na} \cong \frac{\text{Na}^+ + \text{K}^+}{\text{TBW}} - \text{G}_{Na} \Delta [\text{Glucose}]_{p}
\]

Error is a concern, but a slight variation minimizes error (< 5-10%) and is clinically useful to rationalize dysnatremias including pseudohyponatremia:

\[
P_{Na} \cong f_{PW} \frac{\text{Na}^+ + \text{K}^+}{\text{TBW}} - (\text{G}_{Na} \Delta [\text{Glucose}]_{p}) - P_{K}
\]

Notably, this shorthand equation may be derived if one assumes plasma and total body tonicity equality (m_{osm} = 1 and b_{osm} = 0) with no osmotically inactive Na\(^+\) and K\(^+\) (\text{Na}^+_e + \text{K}^+_e = 0) and glucose representing the only non-Na\(^+\) and K\(^+\) effective osmole glucose (\[\Sigma \text{TB} [s]\_i * ((\phi_{bi} - \phi_{pi}/V_{Di}) + \phi_{pNa+K}) = \text{G}_{Na} \Delta [\text{Glucose}]_{p}\)). As noted earlier, error may be significant if the y-intercept of the total body cation relationship changes with pathologic states (i.e. >> 250 mEq).

6. Estimation of Fixed Charge Density and \(\Pi_{GAG}\) for Skin Na\(^+\) Storage

If 20-40 mEq/L of excess skin [Na\(^+\)] is completely stored in the interstitium, then the interstitial [Na\(^+\)] concentration must rise by a similar amount if we assume skin extracellular water is the vast majority of total skin water (>90%). Based on studies in cartilage,\(^ {44}\) an ideal Donnan equilibrium is likely to apply allowing a calculation of fixed charge density (FCD):

\[
[\text{Na}^+]_{GAG} * [\text{Cl}^-]_{GAG} = [\text{Na}^+]_{if} * [\text{Cl}^-]_{if}
\]

\[
\text{FCD} = [\text{Na}^+]_{GAG} - [\text{Cl}^-]_{GAG}
\]

where \(GAG\) and \(if\) represent ionic concentrations in the GAG and bathing interstitial fluid compartments respectively. If we let [Na\(^+\)]_{if} and [Cl\(^-\)]_{if} = 150 mEq/L (assuming equivalency of ions) and set [Na\(^+\)]_{GAG} to 170-190 (i.e. 20-40 mEq/L > than 150 mEq/L), then FCD = 37.5 – 71.1 mEq/L. \(\Pi_{GAG}\) may be estimated assuming
ideal Donnan behavior: \[\Pi_{\text{GAG}} = \varphi_{\text{NaCl}} \times RT \times ([\text{Na}^+]_{\text{GAG}} + [\text{Cl}^-]_{\text{GAG}} - 2 [\text{Na}^+]_{\text{it}} + [\text{GAG}])\]

Assume \(([\text{Na}^+]_{\text{GAG}} + [\text{Cl}^-]_{\text{GAG}}) \gg [\text{GAG}]\) and \(\varphi_{\text{NaCl}}\) at 0.14 M \(\cong 0.93\)

\[\Pi_{\text{GAG}} = 45 - 160 \text{ mm Hg} \ (2.5 - 8 \text{ mOsm/kg})\]

Alternatively, we can utilize an empirical equation derived from various proteoglycan preparations: \(^{46, 47}\)

\[\Pi_{\text{GAG}} \ (\text{mm Hg}) = 760 \ [3.51 \times (\text{FCD}/1000) + 19.3 \times (\text{FCD}/1000)^2]\]

\[\Pi_{\text{GAG}} \ (\text{mm Hg}) = 120 - 263 \text{ mm Hg} \ (6.2 - 13.6 \text{ mOsm/kg})\]
### Supplemental Table 1. Plasma Protein $MW_{av}$ Calculations in Low Albumin States.

$f_{alb} = \text{albumin fraction of total protein (plasma albumin ÷ total protein)}$. Normal patient limiting slopes adapted from references 14 and 11. Congenital analbuminemia data were derived using the data in reference 15: $f_1 = 0.13$ (normal ~ 0.033), $MW_{α1} = 45$ kD; $f_2 = 0.25$ (normal ~ 0.092), $MW_{α2} = 115$ kD; $f_3 = 0.32$ (normal ~ 0.12), $MW_β = 125$ kD; and, $f_4 = 0.3$ (normal ~ 0.16), $MW_γ = 250$ kD (normal ~ 150 kD). The relative distribution of globulin fractions is significantly altered, but the molecular weights are normal except for $MW_γ$. Analbuminemic rat globulin slope estimated from reference 13. Nephrotic patient data calculated from reference 12 assuming $f_{alb} = 0.4$ (mean value of nephrotic cohort) and total protein limiting slope = 1.65 (determined by the authors). The resulting total protein slope and $MW_{av}$ for $f_{alb} = 0.2$ in nephrotic syndrome also agrees with data from reference 16. Hypoalbuminemia data derived by assuming globulin limiting slope is slightly lower than congenital analbuminemia as serum protein profile is similar in these states.  

<table>
<thead>
<tr>
<th>Condition</th>
<th>Plasma Protein Fraction</th>
<th>$dΠ / dc (c = 0)$</th>
<th>$MW_{av}$ (kD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Total Protein</td>
<td>2.1</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>2.8</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Globulin</td>
<td>1.3</td>
<td>149</td>
</tr>
<tr>
<td>Congenital Analbuminemia</td>
<td>Globulin (&amp; total protein)</td>
<td>1.7</td>
<td>113</td>
</tr>
<tr>
<td>Nagase Analbuminemic Rat</td>
<td>Globulin (&amp; total protein)</td>
<td>2</td>
<td>97</td>
</tr>
<tr>
<td>Nephrotic Patients</td>
<td>Albumin</td>
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<td>69</td>
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<td></td>
<td>Globulin</td>
<td>0.9</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>Total Protein ($f_{alb} = 0.4$)</td>
<td>1.65</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>Total Protein ($f_{alb} = 0.3$)</td>
<td>1.47</td>
<td>132</td>
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<tr>
<td></td>
<td>Total Protein ($f_{alb} = 0.2$)</td>
<td>1.28</td>
<td>151</td>
</tr>
<tr>
<td>Inflammatory Hypoalbuminemia</td>
<td>Albumin</td>
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<td>69</td>
</tr>
<tr>
<td></td>
<td>Globulin</td>
<td>1.6</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>Total Protein ($f_{alb} = 0.4$)</td>
<td>2.1</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Total Protein ($f_{alb} = 0.3$)</td>
<td>2</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Total Protein ($f_{alb} = 0.2$)</td>
<td>1.84</td>
<td>105</td>
</tr>
</tbody>
</table>
Supplemental Table 2. Linear Regression Analysis of Plasma and Body Tonicity Relationships. The Edelman and pooled data set of Edelman,\textsuperscript{27} Boling,\textsuperscript{28} and Olesen\textsuperscript{30} were analyzed by ordinary linear regression, Deming regression (corrected for dilution bias), and Passing-Bablok regression (non-parametric) using Analyse-It for Microsoft Excel (version 2.20; Analyse-it Software, Ltd.; Leeds, U.K.). Values indicate means with 95% C.I. in parentheses. The $P_{Na} + P_{K}$ relationship clearly diverges amongst linear regression algorithms, while the $Na^+e + K^+e$ relationship demonstrates similar estimates regardless of regression technique with improved confidence intervals particularly for the pooled data set. Using the $Na^+e + K^+e$ relationship for the pooled data set and averaging the three regression estimates yields a slope of 0.97 (2 out of the 3 estimates suggest the slope is statistically less than 1) and a y-intercept of 250 mEq.

<table>
<thead>
<tr>
<th>Relationship: Molal $P_{Na} + P_{K} = \text{Slope} \times [(Na^+e + K^+e) \times TBW] + \text{Y-intercept}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Edelman Data Set</strong></td>
</tr>
<tr>
<td><strong>Slope</strong></td>
</tr>
<tr>
<td>0.92</td>
</tr>
<tr>
<td>7.11</td>
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</tbody>
</table>

| **Pooled Data Set**                                      | **Deming** | **Passing-Bablok** |
|---------------------------------------------------------------|
| **Slope**  | **Y-intercept** | **Slope**  | **Y-Intercept** | **Slope**  | **Y-Intercept** |
| 0.69      | (0.59 – 0.8)   | 0.98      | 1.53            | 0.99      | -1.17           |
| 44.0      | (28.7 – 59.4)  | (0.83 – 1.13)| (-21.2 – 24.2)| (0.87 – 1.12)| (-20.5 – 17.7)|

<table>
<thead>
<tr>
<th>Relationship: $Na^+e + K^+e = \text{Slope} \times [(\text{Molal } P_{Na} + P_{K}) \times TBW] + \text{Y-intercept}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Edelman Data Set</strong></td>
</tr>
<tr>
<td><strong>Slope</strong></td>
</tr>
<tr>
<td>0.985</td>
</tr>
<tr>
<td>230</td>
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</tbody>
</table>

| **Pooled Data Set**                                      | **Deming** | **Passing-Bablok** |
|---------------------------------------------------------------|
| **Slope**  | **Y-intercept** | **Slope**  | **Y-Intercept** | **Slope**  | **Y-Intercept** |
| 0.96      | (0.93 – 0.98)   | 0.97      | 232             | 0.98      | 222             |
| 299       | (167 – 431)     | (0.95 – 0.99)| (113 – 350)    | (0.95 – 1) | (81 – 346) |
**Supplemental Table 3. Exchangeable Excess Cation Balance.** Exchangeable excess cation concentration was calculated as estimated tissue exchangeable cation concentration (see Table 2) minus 154 mEq/L (estimated plasma cation concentration). Excess cation in mEq/kg was then calculated by multiplying excess concentration by compartment volume. Since the interstitial and transcellular compartments are the only known compartments with cation concentrations less than plasma, these were assumed to raise the slope (Δm), while all other compartments were assumed to modify the y-intercept of the P$_{Na}$ relationship. The estimates are not exactly equal to the regression estimates from Supplemental Table 2, but are within the estimate error.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Excess Cation Concentration (mEq/L H$_2$O)</th>
<th>Volume (L/kg body weight)</th>
<th>Excess Cation (mEq/kg body weight)</th>
<th>Mechanism for Cation Excess</th>
<th>Δ m$_{osm}$</th>
<th>Δ Y-Intercept (mEq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial/Transcellular</td>
<td>-4</td>
<td>0.13</td>
<td>-0.5</td>
<td>Hydrostatic Pressure</td>
<td>+0.006</td>
<td>-</td>
</tr>
<tr>
<td>Cartilage</td>
<td>100</td>
<td>0.012</td>
<td>1.44</td>
<td>Hydrostatic Pressure</td>
<td>-</td>
<td>-88</td>
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<tr>
<td>Bone</td>
<td>250</td>
<td>0.012</td>
<td>3.6</td>
<td>Osmotic Inactivity</td>
<td>-</td>
<td>-210</td>
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<tr>
<td>Intracellular</td>
<td>2</td>
<td>0.33</td>
<td>0.66</td>
<td>Osmotic Inactivity</td>
<td>-</td>
<td>-48</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>0.6</strong></td>
<td><strong>3.7</strong></td>
<td></td>
<td><strong>1.006</strong></td>
<td><strong>-346</strong></td>
</tr>
</tbody>
</table>
References


