Water and Electrolyte Content of Normal and Hypertensive Arteries in Dogs

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Water and electrolytes are important constituents of the blood vessel wall since they affect the chemical environment of vascular smooth muscle and connective tissue as well as the mechanical properties of the wall itself. For example, in hypertension the water and electrolyte contents of blood vessels have been found to be altered.\(^1\)\(^-\)\(^3\) While the dog is frequently utilized in cardiovascular research, quantitative information regarding normal distribution of water and electrolyte contents along the canine arterial tree is meager and few studies have involved more than one or two sites.\(^4\)\(^-\)\(^12\) The primary objective of this investigation was to establish values for the normal water and electrolyte contents along the main arterial tree in dogs and to determine how the normal electrolyte pattern was altered in renal hypertension.

Methods

Mongrel dogs for this study were selected on the basis of size (over 12 kg), apparent good health, and middle age. All were sacrificed under anesthesia (1:1 mixture of 5.5 diallylbarbituric acid and pentobarbital 25 mg/kg) so that portions of the arterial tree could be obtained for analysis. The following sites were analyzed: ascending aorta, 0.5 cm distal to valves; descending aorta, proximal to the first intercostals; aorta, 2 cm above the diaphragm; aorta, proximal to the bifurcation; femoral artery; popliteal artery; carotid artery; lingual artery; main pulmonary artery; and mesenteric arteries of 0.5 to 1 mm diameter. Approximately 30 minutes were required for the complete dissection. The order in which segments were removed was varied from experiment to experiment.

Whole arterial segments including adventitia, but no loose connective tissue, were quickly dissected, lightly blotted of adhering blood and placed in tared stoppered tubes. The fresh samples were weighed, oven-dried for 24 hours at 96°C, and reweighed. The weight difference was taken as the total water. The solids were extracted with ethyl ether, and weighed to determine the fat free solids. Electrolytes were extracted for three days using 0.75 normal HNO\(_3\). This extraction technique yielded at least 99% recovery when known quantities of electrolyte were added to tissue samples. The sodium and potassium in the extract were analyzed with a flame photometer as described by Raymond.\(^13\) Chloride from the same extract was determined in duplicate using a Cotlove chloride titrator. Serum electrolytes were analyzed using the same techniques. Serum solid matter was estimated from the measurement of the specific gravity using a drop technique into copper sulfate solution.\(^14\) Tissue water and electrolytes were expressed per kilogram fresh tissue wet weight (kg wet wt) and kilogram fat free solid (kg fat free solid).

An analysis of variance was done on 88 determinations (11 dogs and 8 sites per dog) for the constituents, water, sodium, potassium, and chloride. In the analyses of each constituent the variation associated with differences among dogs and differences among sites was estimated and the discrepancy between this and the total variation was used to estimate the experimental error.\(^15\) These analyses of variance gave the following standard deviations: water, 11.3 g/kg wet wt; sodium, 6.8 meq/kg wet wt; potassium, 3.6 meq/kg wet wt; chloride, 3.3 meq/kg wet wt.

In addition, the inulin space of the vascular wall segments was determined by an in vitro technique. If the arterial wall thickness was greater than 1 mm, a strip 1 mm wide was cut from the trimmed open vessel perpendicular to the long axis. The slices were equilibrated by incubating for three hours in a standard Kreb's solution containing 0.75% alkali stable inulin at 37.5°C with a gas phase of 95% O\(_2\) and 5% CO\(_2\).
The slices were then lightly blotted with filter paper and weighed. The inulin was extracted 22 hours at 4°C into 5 ml of Kreb's solution which contained no carbohydrate. Analyses were done on duplicate samples of the extract using the method of Higashi. The alkali stable inulin used in these experiments was prepared by the method of Walser. The tissue blank was equivalent to 0.4 µg inulin per tube or approximately 0.5% of the total. An analysis of variance performed on 63 observations (nine dogs and seven sites) of inulin determinations yielded a standard deviation of 33 g/kg wet wt.

The inulin space was calculated with the assumption that the quantity of inulin taken up by the slices was distributed in the tissue water in the same concentration as the bathing media. The following relation was used for this calculation:

\[ I_{sp} = \frac{I}{[I]_o} \]

where \( I_{sp} \) is the inulin space, \( I \) is the quantity of inulin taken up, and \([I]_o\) is the concentration of inulin in the bath solution. The cell water was estimated by taking the difference between the total water and the inulin space. While this is not a direct measurement, the method is frequently used to estimate cellular water content.

The concentrations of electrolytes in the estimated cellular water were calculated by correcting the total electrolyte content for the amount estimated to be present in the inulin space. The electrolyte concentration in the inulin space was taken as that of a plasma ultrafiltrate, correcting concentrations in plasma water for the Donnan effect. The following relation was used for this calculation:

\[ [A]_e = \frac{A_t - [A]_o I_{sp}}{W_t - I_{sp}} \]

where: \([A]_e\) is the concentration of A (sodium, potassium, and chloride) in the estimated cell water, \(A_t\) is the total quantity of A, \([A]_o\) is the concentration of A in the inulin permeable water, \(W_t\) is the total water and \(I_{sp}\) is the inulin space.

In a related study the water and electrolyte content of four sites along the aorta were measured in eight dogs made hypertensive by wrapping their kidneys in cellophane. The selection and treatment of animals were the same in this study as in the normal group, the only difference being due to alterations within the animal following kidney wrapping. The hypertensive group was sacrificed with the same anesthesia and the vessels analyzed as in the normal group. Blood pressures were measured by direct arterial puncture and the hypertensive animals had a sustained mean blood pressure elevation of at least 30 mm Hg for a minimum of four weeks. The normal and hypertensive groups were compared using an unpaired Student's t-test.

Normal total water, cell water, potassium, sodium, and chloride content for eight arterial sites plotted at their relative anatomical spacing. The sites are: lingual artery, (LING); carotid artery, (CAR); ascending aorta, (A.A.); descending aorta, (D.A.); aorta 2 cm above the diaphragm, (DIA); bifurcation of aorta, (BIF); femoral artery, (FEM); popliteal artery, (POP). Vertical bars represent one D value. Two adjacent or nonadjacent sites that do not have overlapping vertical bars are significantly different (P<0.01).
Results

In order to characterize statistically the water and electrolyte differences from site-to-site along the arterial tree, it was most appropriate to utilize only data from animals in which all the sites were analyzed. This procedure contrasts site-to-site rather than animal-to-animal differences. In each such statistical analysis, the average value for each arterial site was compared pairwise to all other sites. The statistical method of J. W. Tukey as described by Snedecor was used. It is based on the calculation of a D value dependent on the standard deviation, degrees of freedom, number of sites compared, and desired confidence level. When averages at two arterial sites differ by more than D the difference is said to be statistically significant. For these analyses the confidence level used was P < 0.01.

The mean values of the water and electrolyte contents (11 dogs) and the estimated cellular water (9 dogs) of arterial samples are given in figure 1. The ordinate is divided into five different scales for the five vessel constituents. The abscissa indicates the sampled sites plotted at distances proportional to their anatomical separation as measured along the arterial tree. The vertical bars indicate one D value. Thus data from two sites which do not provide overlapping vertical bars are significantly different (P < 0.01). For example: the water content of the ascending and descending aorta are not significantly different, but the water contents of these sites were significantly greater than all other, more peripheral sites.

The total water content, the estimated cell water volume, and the potassium content of the arteries sampled, showed similar trends. The ascending aorta had the highest values and more distal portions of the arterial tree toward the head and hind limb exhibited decreasing values of water and potassium.

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of dogs</th>
<th>Water (g/kg wet wt)</th>
<th>Electrolyte (meq/kg wet wt)</th>
<th>Content (meq/kg fat free solid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>Lingual</td>
<td>11</td>
<td>718.7</td>
<td>101.0</td>
<td>46.4</td>
</tr>
<tr>
<td>Carotid</td>
<td>14</td>
<td>721.3</td>
<td>±3.8</td>
<td>±4.3</td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>18</td>
<td>744.8</td>
<td>±3.5</td>
<td>±1.4</td>
</tr>
<tr>
<td>Descending aorta</td>
<td>18</td>
<td>738.1</td>
<td>±3.4</td>
<td>±1.4</td>
</tr>
<tr>
<td>Diaphragm aorta</td>
<td>18</td>
<td>712.8</td>
<td>±2.8</td>
<td>±1.4</td>
</tr>
<tr>
<td>Femoral</td>
<td>18</td>
<td>721.0</td>
<td>±3.2</td>
<td>±1.8</td>
</tr>
<tr>
<td>Popliteal</td>
<td>18</td>
<td>698.8</td>
<td>±4.7</td>
<td>±2.0</td>
</tr>
<tr>
<td>Main pulmonary</td>
<td>11</td>
<td>712.7</td>
<td>±5.0</td>
<td>±2.5</td>
</tr>
<tr>
<td>Mesentery</td>
<td>11</td>
<td>779.8</td>
<td>±3.4</td>
<td>±1.8</td>
</tr>
<tr>
<td>Serum</td>
<td>18</td>
<td>941</td>
<td>±1</td>
<td>±2</td>
</tr>
</tbody>
</table>

±Standard error of mean.
Spaces and Calculated Ion Concentrations in Cell Water

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of dogs</th>
<th>Inulin space g/kg wet wt</th>
<th>Cell water g/kg wet wt</th>
<th>Electrolyte concentration meq/kg</th>
<th>Chloride space g/kg wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid</td>
<td>8</td>
<td>406</td>
<td>315</td>
<td>102 ±7 125 ±8 Cl ±5</td>
<td>619 ±9</td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>9</td>
<td>378 ±8</td>
<td>368 ±13</td>
<td>70 ±4 129 ±8 Cl ±2</td>
<td>545 ±7</td>
</tr>
<tr>
<td>Descending aorta</td>
<td>9</td>
<td>389 ±10</td>
<td>351 ±17</td>
<td>77 ±9 132 ±6 Cl ±4</td>
<td>560 ±7</td>
</tr>
<tr>
<td>Diaphragm aorta</td>
<td>9</td>
<td>401 ±16</td>
<td>312 ±16</td>
<td>72 ±9 140 ±12 Cl ±4</td>
<td>552 ±8</td>
</tr>
<tr>
<td>Bifurcation of aorta</td>
<td>9</td>
<td>471 ±13</td>
<td>255 ±14</td>
<td>96 ±10 143 ±8 Cl ±4</td>
<td>617 ±8</td>
</tr>
<tr>
<td>Femoral</td>
<td>9</td>
<td>404 ±12</td>
<td>302 ±14</td>
<td>113 ±8 103 ±3 Cl ±3</td>
<td>613 ±9</td>
</tr>
<tr>
<td>Popliteal</td>
<td>9</td>
<td>479 ±14</td>
<td>236 ±14</td>
<td>110 ±8 152 ±13 Cl ±8</td>
<td>589 ±13</td>
</tr>
<tr>
<td>Main pulmonary</td>
<td>9</td>
<td>478 ±13</td>
<td>300 ±13</td>
<td>63 ±5 159 ±8 Cl ±3</td>
<td>580 ±6</td>
</tr>
</tbody>
</table>

±Standard error of mean.

TABLE 2

As with other smooth muscle tissues the total intracellular cation concentration is greater than the cation concentration in the extracellular fluid,18 indicating that some fraction of intracellular cations are osmotically less active.

The water and electrolyte content of four sites along the aorta of eight hypertensive dogs are summarized in table 3. The water and sodium content of hypertensive vessels were significantly elevated above the normal values listed in table 1 for all sites along the aorta (P<0.001). The chloride contents of...
the hypertensive vessels were not significantly increased when expressed per kg wet weight, but chloride in the proximal aorta was elevated when expressed per kg fat free solids. There were no significant changes in vessel potassium content associated with renal hypertension. The trend that was seen in normal dogs of decreasing water and potassium content proceeding distally along the aorta was also qualitatively evident in hypertensive animals.

**Discussion**

These analyses show that from the ascending aorta to the femoral and carotid arteries the potassium and water contents decreased, while the sodium and chloride contents increased. This was observed in the normal and hypertensive animals. Analyses of three higher order branches of the arterial tree (mesentery, lingual, popliteal) indicate that these trends may not persist along the smaller vessels. Since cells have a higher potassium and water content than connective tissue which is rich in sodium and chloride, a simple explanation of the trend in water and electrolyte content along the arterial tree is that the relative amounts of cells and connective tissue vary among these sites. Thus, the upper aorta contains a higher proportion of cell content than the more distal vessels to the femoral and carotid arteries. These trends may not persist along the entire vascular tree as indicated by analysis of higher order branches.

Inulin was used to identify the rapidly equilibrating tissue water in order to evaluate water distribution within the vessel wall. The inulin spaces in the proximal aorta were smaller than those in the more distal sites. This is consistent with the findings and explanation presented. These values for inulin spaces in dog arteries are similar to other values in the literature. If one makes the assumption that the total water minus the inulin space is mostly intracellular water then this is further evidence that the ascending aorta has a larger proportion of cellular (smooth muscle) material than the more distal sites along the arterial tree to the femoral and carotid arteries. Such estimates of intracellular water are probably valid for tissues such as myometrium and *taenia coli* which are almost completely smooth muscle. However, for connective tissue such as tendon, inulin space determinations tend to underestimate the non-cellular water unless equilibrated for prolonged periods. Blood vessels contain a relatively high proportion of connective tissue; approximately 50% of the dry solids are composed of collagen and elastin. While the presence of connective tissue may tend to

**TABLE 3**

*Water and Electrolyte Content of Hypertensive Dog Arteries*

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of dogs</th>
<th>Water g/kg wet wt</th>
<th>Electrolyte Na meq/kg wet wt</th>
<th>Electrolyte K meq/kg wet wt</th>
<th>Electrolyte Cl meq/kg wet wt</th>
<th>Content Na meq/kg fat free solid</th>
<th>Content K meq/kg fat free solid</th>
<th>Content Cl meq/kg fat free solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending aorta</td>
<td>8</td>
<td>±2.9</td>
<td>94.0±1</td>
<td>45.0</td>
<td>71.4</td>
<td>420±1</td>
<td>201±1</td>
<td>319±1</td>
</tr>
<tr>
<td>Descending aorta</td>
<td>8</td>
<td>±1.1</td>
<td>89.8±1</td>
<td>46.2</td>
<td>67.5</td>
<td>392±1</td>
<td>202±1</td>
<td>295±1</td>
</tr>
<tr>
<td>Diaphragm aorta</td>
<td>8</td>
<td>±1.8</td>
<td>88.8*1</td>
<td>42.9</td>
<td>65.0</td>
<td>380±1</td>
<td>174±1</td>
<td>263*1</td>
</tr>
<tr>
<td>Bifurcation of aorta</td>
<td>7</td>
<td>±1.5</td>
<td>86.8±1</td>
<td>32.9</td>
<td>71.7</td>
<td>390*1</td>
<td>132±1</td>
<td>288</td>
</tr>
<tr>
<td>Serum</td>
<td>8</td>
<td>±1.6</td>
<td>96.8±1</td>
<td>32.9</td>
<td>71.7</td>
<td>390*1</td>
<td>132±1</td>
<td>288</td>
</tr>
</tbody>
</table>

±Standard error of mean.

*0.001 < P < 0.01.

1P < 0.001.
increase the estimated cell water volume for any one site, the connective tissue elements should not substantially affect the differences between sites noted in the pairwise comparisons made along the arterial tree.

Differences in the vessel wall properties within the vascular tree of dogs have been reported. The oxygen uptake (per dry weight) of slices and homogenates of the aortic arch and thoracic aorta were found to be higher than those of abdominal aorta segments. Also, adenosine triphosphate activity of the dog aorta was found to be twice that in the carotid and femoral arteries. Furthermore, the total nitrogen per dry solid was relatively constant along the aorta, but the alkali soluble nitrogen (mostly protein other than collagen and elastin) was highest at the arch and lowest in the abdominal segment with an intermediate value for the thoracic aorta.

There is also evidence that the relative amounts of connective tissue types are different in various sites along the vascular system. From the ascending aorta to the level of the diaphragm, elastin composed about 50% of the connective tissue, while elastin constitutes about 30% of the major connective tissue elements in the more peripheral sites. The mucopolysaccharide content (hexosamine) was found to be greatest at the arch, least in the abdominal segment with an intermediate value for the thoracic aorta.

As stated above, the simplest explanation to account for our findings is that there are differences in the relative amount of cells and connective tissue along the arterial tree. It should be noted that there are other possible explanations whose basis is still to be firmly established, e.g., selective ion association with tissue protein. Evidence has been presented concerning ion association with mucopolysaccharides and this has been postulated as being a significant mechanism of ion accumulation in dog carotids. Concepts have also been presented concerning intracellular ionic association within a fixed charge system and sodium-potassium selectivity in such a system.

The water and electrolyte contents of the arterial walls are also of significance in diseases of the vascular system. In this study there was a constant elevation in water content along the aorta associated with renal hypertension. Sodium was generally elevated, but was greatest in the proximal, apparently more cellular, sites. The largest and most significant changes in chloride were also noted in these sites. The net increase of sodium was greater than chloride for all sites compared and was greater than that predicted if the increase of water and electrolyte were the result of isotonic edema fluid. The plasma electrolyte levels were not significantly altered in the hypertensive dogs, thus indicating local alteration of the ion accumulating mechanisms of the arterial wall. The increase of water content seen along the aorta in this study was also manifest in the femoral arteries of the same hypertensive dogs.

The relation of water and electrolyte metabolism to vascular function has been emphasized by several authors. The water content itself may cause the vessel wall to become stiffer. Furthermore, a thicker wall results in a decreased internal radius and radius-wall thickness ratio, each tending to increase wall stiffness. Changes in electrolyte concentration may also alter functional properties of smooth muscle.

**Summary**

This study was designed to establish values for the water and electrolyte content along the arterial tree in the dog under normal conditions and to determine how the normal electrolyte pattern was altered in renal hypertension.

Determinations of vessel wall water, potassium, sodium, and chloride contents have shown that the water and electrolyte contents from the various sites along the arterial tree differ significantly from each other. Total water and potassium contents decrease progressively from the ascending aorta to the femoral and carotid sites, while the sodium and chloride contents showed the opposite trend. These differences were significant statistically. These trends may not persist along the
entire vascular tree to very small arteries, as indicated by analysis of mesenteric arterial samples. Studies of vessel wall inulin space supported the view that these differences result from the relative distribution of cellular and noncellular material; thus the proximal aorta contains a relatively greater proportion of cellular (smooth muscle) material than more distal sites such as the femoral and carotid arteries. A review of the literature further supports these findings.

In experimentally induced renal hypertension the same trends persist along the aorta, but the total water and sodium contents are significantly elevated at all sites.

References
Water and Electrolyte Content of Normal and Hypertensive Arteries in Dogs
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