Rapid Measurement of Serum Water to Assess Pseudohyponatremia

Sherry Faye¹ and R. B. Payne²

Pseudohyponatremia is caused by an increased serum protein or lipid concentration producing a "space-occupying lesion" in serum water. Its presence and magnitude must be assessed in hyponatremic patients with, for example, paraproteinemias or hyperlipemics. In the absence of a direct-reading ion-selective electrode system, a method for measuring the water content of serum is required. We describe two rapid methods for measuring the diffusible water of serum: osmometry before and after dilution and chloride measurement before and after ultrafiltration. Either of these methods allows the true sodium status of a patient's serum to be determined.

Additional Keyphrases: electrolytes · osmometry · ultrafiltration · paraproteinemia · hyperlipemia · ion-selective electrodes

In health, water accounts for 93% of serum volume, only 7% being occupied by dissolved solids. Serum water may be considerably decreased in paraproteinemia or severe hyperlipemia, so that the concentration of a solute such as sodium is decreased per unit volume of serum but may be normal per unit volume of water. For example, if serum water is decreased to 80%, a low sodium concentration of 120 mmol/L measured by a flame photometer or an indirect ion-selective electrode (ISE) system represents a concentration of 150 mmol/L in serum water and thus a normal concentration of 140 mmol/L in serum with a normal water content of 93%. Unnecessary treatment of pseudohyponatremia has caused morbidity and contributed to death (1). A decrease in serum water content does not always cause hyponatremia; it may, though less often, mask true hyponatremia and produce "pseudonatremia" (2, 3).

The use of a direct-reading ISE system to measure sodium concentration in serum water in such patients has been advocated (4–6) but has some disadvantages that will be discussed later. The alternative approach is to measure the diffusible water content of serum so that the sodium concentration in serum water and thus in normal whole serum can be calculated from the measured sodium concentration. Methods based on the measurement of the concentration in serum of either protein alone (7–10) or lipid alone (11) cannot be used for all patients. We have compared three methods for measuring serum water that can be applied in both hyperproteinemias and hyperlipemias.

Methods

Analytical Methods

Total protein concentration was determined by a manual biuret method with a within-batch analytical CV of 3.4%.

Total lipid concentration was measured with a Merco test total lipids kit with a within-batch analytical CV of 5.8%. Amicon Centriflo membrane cones, type CF25, were used for ultrafiltration. Before use, the cones were soaked in distilled water for at least an hour and then blotted and centrifuged to remove excess water. They were cuffed with plastic film during centrifugation at room temperature to minimize evaporation, and the first few milliliters of ultrafiltrate were discarded to avoid dilution with residual cone water. In later experiments the Amicon Centrifree Micropartition System was used for ultrafiltration. Chloride concentration was measured with a Corning-Eel 120 Chloride Meter or, in later experiments, a Monitor Parallel discretionary analyzer. The within-batch analytical CVs for single measurements were 0.85% for the chloride meter and 1.53% for the Parallel analyzer at normal serum concentrations. Osmolality was measured with a Precision Instruments Osmette-S osmometer, an Advanced Instruments Model 64131 osmometer, or a Roebling osmometer. Values measured on the three instruments did not differ significantly and the within-batch analytical CVs for single measurements ranged from 0.25% to 0.65%. The pH of serum was determined with an IL 313S blood gas analyzer.

Calculations

Serum water was calculated from the total lipid and total protein concentrations in serum by using the equation of Waugh (12):

\[
\text{Serum water (g/dL)} = 99.1 - (1.03 \times \text{lipid concn}) - (0.73 \times \text{protein concn})
\]

where total lipid and total protein are both expressed in g/dL. This method is based on the average specific volumes occupied by known weights of serum proteins as determined by drying and weighing, and of lipids by derivation from density measurements. The method had a mean within-batch analytical CV of only 0.2%; it was small in spite of the greater analytical imprecisions of the two measurements because there is a large constant in the equation.

We calculated serum water from the means of replicate chloride measurements in serum and its ultrafiltrate by using the equation:

\[
\text{Serum water (g/dL)} = \left(\frac{\text{chloride concn in serum}}{\text{chloride concn in ultrafiltrate}}\right) \times 100
\]

This method is based on a principle described by Little and Payne (13): because chloride binding to serum proteins is negligible at pH values >7.0 (14), the serum ultrafiltrate chloride concentration is the same as the chloride concentration in the diffusible water phase of serum. The means of triplicate measurements had a mean within-batch analytical CV of 0.9%.

We measured serum water by osmometry, using the method of Rawles (15). We preferred this method to an earlier method based on the same principle requiring osmolality measurements before and after the addition of an exact amount of anhydrous sodium chloride (16) because of
its technical simplicity. Serum was diluted with an equal volume of water and replicate measurements of the osmolality of the original and diluted sera were made to determine mean values. The calculation is:

\[
\text{Serum water (\%)} = \frac{\text{osmolality of diluted serum - osmolality of diluted osmolality}}{100}
\]

The basis of this method is that, when a solution containing a "space-occupying lesion" caused by proteins and lipids is diluted with an equal volume of water, the reduction in the concentration of crystalloids dissolved in the aqueous phase, and therefore the reduction in the measured osmolality, is greater than that predicted from the volumes mixed so that the true volumes of aqueous phase mixed, and therefore serum water, can be determined. With use of the means of duplicate measurements, the method had a mean within-batch analytical CV of 1.3%.

Results

Preliminary Investigation of the Chloride Method

A pooled serum was ultrafiltered in a Centriflo cone and the total protein and chloride concentrations of successive aliquots of the ultrafiltrate and retentate were measured. The concentrations of chloride in successive filtrates did not differ significantly, and they were protein-free. There was a close negative linear correlation between the concentrations in the retentate of protein, which increased from 60 to 140 g/L, and of chloride (\(r = -0.993\)). The Deming regression equation for the retentate values was:

\[
\text{Chloride (mmol/L)} = 114.3 - (\text{total protein (in g/L)} \times 0.178)
\]

The intercept value agreed well with the ultrafiltrate chloride concentration of 114.1 mmol/L. The constancy of filtrate chloride concentration was confirmed by prolonged ultrafiltration of a further six sera (Table 1). The pH values for all pools of laboratory sera examined exceeded 7.72. We concluded that there is negligible binding of chloride to protein in separated sera at the pH reached after exposure to room air, as predicted from the observations of Carr (14).

Recovery Experiments

Pooled serum from blood donors was centrifuged in a number of Centriflo cones and the ultrafilters and retentates were pooled. The pooled retentate had nearly twice the protein concentration of the original pool and therefore had a low water content. Retentate was mixed with ultrafiltrates in the proportions 2:1, 1:1, 2:3, and 1:2, and the serum water concentrations of the retentate and its dilutions with ultrafiltrate were determined eight to 10 times by each of the three methods. Water accounted for was calculated as the difference between the mean percentage water in the concentrated serum and the product of the mean percentage water in the dilution and the relative volume of ultrafiltrate added. Least-squares regression analysis of serum water measured vs serum water (ultrafiltrate) added gave correlation coefficients >0.9997 in each case and the regression equations were:

\[
\begin{align*}
\text{Water recovered (Waugh)} &= (0.9901 \times \text{water added}) - 0.0580 \\
\text{Water recovered (chloride)} &= (0.9967 \times \text{water added}) - 1.0293 \\
\text{Water recovered (Rawles)} &= (1.0293 \times \text{water added}) - 0.1420
\end{align*}
\]

The slope, and therefore recovery, by Waugh's protein/lipid equation was significantly less than 1.0 (p < 0.001), that by the chloride method did not differ from 1.0, and that by Rawles' osmolality method was significantly greater than 1.0 (p < 0.001). None of the intercepts differed significantly from zero.

We considered that the slightly lower recovery with Waugh's protein/lipid method could probably be attributed to the fact that, like all such equations, the mean specific volumes of proteins were determined by drying and so took no account of the fact that proteins bind water (17). We had expected both the chloride method and Rawles' osmolality method to measure free diffusible water and therefore to give similar results, so the discrepancy between them was investigated further.

Investigation and Modification of the Osmolality Method

This method assumes that the osmotic activity of crystalloid particles in the aqueous phase is unchanged when the specimen is diluted with water. Water was added to six sera in the proportions 3:7, 4:6, 5:5, 6:4, and 7:3. We calculated serum water from osmolality measurements, using the general case of Rawles' equation:

\[
\text{Serum water (\%)} = \frac{\text{osmolality of diluted serum} - \text{osmolality of diluted osmolality}}{(\text{vol of water/\text{vol of serum}}) \times 100}
\]

The calculated serum water concentrations determined by the osmolality method showed significant increases at dilutions greater than 4:6 (paired t-test, p < 0.05) while the values at the two smallest dilutions did not differ significantly (Figure 1). We concluded that a significant increase in activity took place on dilution of serum but that the effect on serum water by the osmolality method was negligible at the smallest dilutions. We therefore modified Rawles' osmolality method by using a dilution of one part of water to two parts of serum instead of equal volumes and the means of replicate measurements were entered in the modified equation:

\[
\text{Serum water (\%)} = \frac{\text{osmolality of diluted serum} - \text{osmolality of diluted osmolality}}{\frac{3}{2}} \times 100
\]

Comparison of Chloride and Modified Osmolality Methods

We prepared ultrafiltrates from 1 mL of each of 10 patients' sera, using the Centriflo Micropartition System centrifuged in a fixed-angle centrifuge (1700 × g, 60 min, 15 °C). Serum water was calculated from the mean of

| Table 1. Chloride Concentrations in Retentate and Filtrate during Prolonged Ultrafiltration of Six Laboratory Sera |
|---|---|---|---|
| Chloride concentration, mmol/L | 15-min centrifugation | 45-min centrifugation |
| | Retentate | Filtrate | Retentate | Filtrate |
| 97 | 114 | 68 | 114 |
| 97 | 114 | 61 | 114 |
| 98 | 113 | 71 | 114 |
| 98 | 113 | 71 | 114 |
| 95 | 114 | 67 | 114 |
| 96 | 114 | 68 | 114 |
| 95 | 114 | 71 | 114 |
triplicate chloride concentrations measured in a Monitor Parallel analyzer in the original sera and in their ultrafiltrates. Serum water was calculated by the method of Rawles (15) and by our modification of Rawles’ method from the mean of duplicate osmolality measurements. The calculated serum water concentrations did not differ significantly between the chloride method and our modified osmolality method (p = 0.90; paired t-test), but were significantly greater (p = 0.0016) by Rawles’ osmolality method (Table 2).

Can Ultrafiltration Be Used to Measure Serum Water Sodium?

Binding of sodium to serum proteins at pH 7.5 has been reported (18) and would be expected to increase with increasing pH. The pH values of our separated serum specimens all exceeded 7.72. We ultrafiltered eight laboratory sera, using Centriflo systems as above. While the chloride concentrations averaged 8.1 mmol/L higher in the ultrafiltrates than in the whole sera (p <0.001), the sodium concentrations averaged 4.4 mmol/L lower (p <0.01), with means of 139.9 mmol/L in the sera and 135.5 mmol/L in the ultrafiltrates. The mean serum water as calculated by the chloride method was 92.7%, so that on average 10.2% of the sodium in the sera was not filterable. Information on the sodium concentration in ultrafiltrates of serum is thus of no value in the assessment of hyponatremia.

Discussion

Hyponatremia is a common clinical problem (6, 19, 20). Laboratory staff may recognize that the serum sodium concentration of a hyperproteinemic or hyperlipemic sample measured by flame photometry or an indirect ISE system is misleading, but they may not know how to obtain a clinically useful result.

An option that is available to some laboratories is to use a direct ISE system. However, Cowell and McGrady (21) found that two commercial direct ISE analyzers, when used to examine hyponatremic samples with normal concentrations of protein and cholesterol, gave values that were 6 to 7 mmol/L lower than the total serum sodium measured by routine methods, results that are both impossible and potentially dangerous. They eliminated the errors in both instruments by modifying the manufacturers’ aqueous calibrants. International agreement has not yet been reached on either the composition of calibrants or on the method of expressing results—as a concentration in serum water or as a concentration in whole serum with a normal water content (22–25). A third direct ISE system has been found to overestimate sodium in hypoproteinemia, the error reaching 10 mmol/L at a total protein concentration of 40–50 g/L (26).

A superficially attractive alternative to direct ISE measurement of sodium concentration is its measurement by routine methods in a protein- and lipid-free ultrafiltrate. However, we have shown that there is only partial ultrafiltration of the sodium in separated laboratory sera.

We propose that the diffusible water content of serum should be measured directly, either by osmometry before and after dilution or by chloride measurement before and after ultrafiltration. Both methods are simple, rapid, and precise and give good analytical recoveries. We have no information about their absolute accuracy because there is no reference method for diffusible serum water. We suggest that the serum sodium concentration measured by routine methods (if necessary after manual dilution and direct delivery to the measuring unit to avoid short sampling due to the hypertonicity of some paraproteinemica sera) should be divided by the measured plasma water (%) and multiplied by a normal plasma water value of 93%. The reported value will thus be adjusted to that which would be found if the serum water content were normal. This convention was suggested by Broughton et al. for direct-reading ISE systems (25). It simplifies clinical interpretation because the conventional reference range can still be used.

This paper incorporates work that formed part of an MSc in Clinical Biochemistry in the University of Leeds.

Table 2. Serum Water (%) in 10 Laboratory Sera as Measured by Rawles’ Osmolality Method (I), the Modified Osmolality Method (II), and the Chloride Method (III)

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Mean (SD) 95.2 (1.79) 93.0 (1.10) 93.1 (1.03)

References