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Relationship between Direct-Potentiometric and Flame-Photometric Measurement of Sodium in Blood

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The relationship between direct-potentiometric and flame-photometric measurements of sodium has been studied in human sera and other, simpler systems. When water content was varied by using an inert substance (silica), de-ionized sera, or de-ionized individual proteins, the percentage that the flame photometric values were of the direct potentiometric Na^+ values was identical to the measured water content. However, in 35 patients the percentage of Na^+ values was 99.1% and the water content was 92.0%, a discrepancy of 7.1%. De-ionization of sera removed this discrepancy, apparently because of the lower pH of the de-ionized sera. The percentage of flame-photometric to direct-potentiometric Na^+ values varied as a function of pH in pooled sera; in lyophilized and reconstituted sera; and in de-ionized, lyophilized, and reconstituted sera. Four explanations for the discrepancy between the percentage of Na^+ values and the water content are discussed: a calibration problem, a measurement artifact, Na^+ binding, and water binding. At this time there are no definitive data concerning which explanation is correct. We expect that our finding of a pH dependency for the percentage of measured Na^+ values can be used to develop model systems to elucidate the mechanism producing the discrepancy between the percentage of Na^+ values and the water content.

Additional Keyphrases: *variation, source of · effect of pH · ion-selective electrodes · sodium · potentiometry*

In recent years ion-selective electrodes have been increasingly utilized for the measurement of sodium in plasma. In 1981, 29% of the sodium values reported in the survey of the College of American Pathologists were determined with ion-selective electrodes rather than by flame photometry (*1*). Ion-selective electrodes for the analysis of sodium are used via two major approaches (Figure 1). In one, the sample is diluted before analysis (indirect potentiometry), analogous to flame photometry. In the other, the sample is analyzed without dilution (direct potentiometry), analogous to blood pH measurement. When using indirect potentiometry, if the diluent and dilution ratio are properly selected, the activity coefficient and residual liquid junction potential between standards and samples should be matched and the results should reflect total concentration. Therefore, it is not surprising that indirect potentiometry and flame photometry give the same values for sodium in plasma with various instruments (*2–9*).

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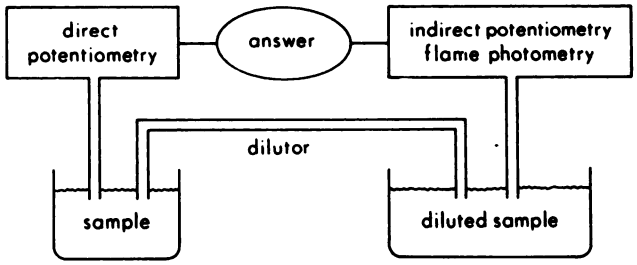

Fig. 1. Schematic representation of undiluted and diluted sampling techniques used by direct potentiometry and indirect potentiometry or flame photometry methods

Direct-potentiometric sodium values are not expected to be the same as flame-photometric values because of the influence of water content as depicted in Figure 2. In clinical conditions such as hyperlipemia (*10–13*) or hyperproteinemia (*14–17*), in which the water content of plasma is decreased, low flame-photometric Na^+ values have been long noted. Under such conditions, direct-potentiometric values are more clinically appropriate (*13, 17*). Given that the water content of normal serum is 91–93%, a technique that utilizes a fixed-volume aliquot (flame photometry or indirect potentiometry) should give values 7–9% lower than a technique that does not involve fixed-volume aliquots (direct potentiometry) when both use standardization with NaCl. This is because direct potentiometry should measure the activity of sodium only in the water phase. However, Na^+ values obtained by direct potentiometry and flame photometry show that flame-photometric values are 95.7 to 98.8% of direct-potentiometric values (*18–22*). The discrepancy between the observed percentage of measured sodium values and that predicted by the measured water content has prompted several papers and letters (*23–30*).

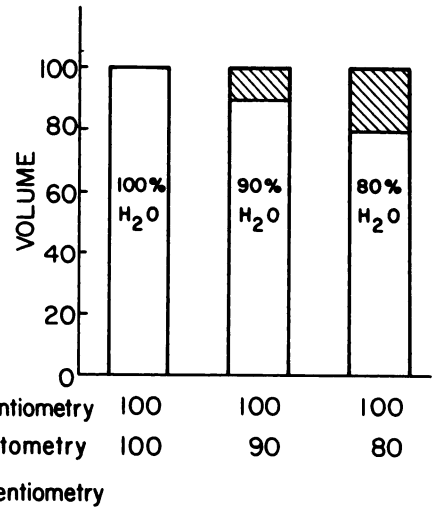

Fig. 2. Predicted influence of water content on sodium measurements for 100 mmol/L NaCl solution

Striped area indicates nonaqueous volume

The purpose of this paper is threefold. First, we hope to clarify the issues that have been raised concerning direct-potentiometric and flame-photometric measurements of sodium. Second, we examine the data for and against the various explanations of the discrepancy between direct-potentiometric and flame-photometric Na^+ values. Third, we present our experience in developing model systems to permit systematic study of the relationship between direct-potentiometric and flame-photometric measurement of sodium in blood.

Materials and Methods

Analytical procedures. Sodium was measured by direct potentiometry with a Nova-1 analyzer (Nova Biomedical Inc., Newton, MA 02164). Where indicated, Na^+ was also measured by direct potentiometry with a Ektachem 400 (Eastman Kodak, Rochester, NY 14650). Sodium was measured by flame photometry with a KLiNa flame photometer (Beckman Instruments Inc., Fullerton, CA 92634), standardized with the same solution used to calibrate the Nova-1 (Nova-A standard: NaCl 140.0 mmol/L, KCl 4.00 mmol/L, and Mg acetate 3 mmol/L, pH 6.8). All analyses for sodium were performed in triplicate, with the samples in random order; the median value is reported.

Water content was measured in duplicate by placing 100 μL of sample (SMI micro/pettor series I; SMI, Berkeley, CA 94710) into a preweighed weighing vial. After reweighing the sample, we heated it at 110 °C overnight, cooled it in a desiccator, and reweighed. Assuming a density of water of 1 g/mL, we calculated the water content of the sample as the loss in weight, mg/100 μL, expressed as a percentage. Protein was measured by the biuret reaction and CO_2 content by an enzymic method, both with an *aca* discrete analyzer (Du Pont Co., Clinical Systems Division, Wilmington, DE 19898). pH was measured at 37 °C with a capillary electrode (Radiometer E5021; Radiometer, Copenhagen, Denmark).

Silica experiments. Silica (1–5 μm particle size, 99.5% between 0.5 and 10 μm; Sigma Chemical Co., St. Louis, MO 63178) was added to the Nova-A standard until the silica represented approximately 70% of the volume. This suspension was constantly stirred, and dilutions were made with the Nova-A standard to give solutions with various water contents. Aliquots for the measurement of water content and sodium by direct potentiometry and flame photometry were taken with the samples constantly stirred. For the flame-photometric determinations, 100-μL aliquots of standard or silica suspensions were diluted to 10 mL with lithium diluent. The diluted samples were centrifuged before analysis to avoid clogging the aspirator–nebulizer. For the direct-potentiometric determinations we disconnected the sample line of the Nova-1 from the sample probe, placed the sample line in the solution, and then reconnected it as usual before the internal standard was sampled. This avoided clogging the sample probe with the samples containing large amounts of silica, and gave the same Na^+ values for aqueous solutions as did the unmodified Nova-1.

De-ionized serum and proteins. Sera from hospitalized patients were pooled and separated into three fractions. One fraction was frozen, one was lyophilized, and the third was de-ionized and lyophilized. The de-ionization procedure consisted of dialysis against stirred water at 4 °C for 48 h in a cellulose dialysis sack (molecular mass cutoff, 12 000 daltons; Fisher Scientific, Pittsburgh, PA 15219) with three changes of water daily. The contents of the dialysis sack were then stirred for 12 h at 4 °C with a mixture of Dowex-2 resin in the chloride form and Dowex 50W in the hydrogen form (both from Sigma Chemical Co.) and then filtered. This resin treatment was performed three times over a 48-h period. After the last filtration the sample was lyophilized. Lyophilized sera were reconstituted to a protein concentration of ~250 g/L with water or Tris buffer (100 mmol/L, pH 7.5). The de-ionized and lyophilized sera were reconstituted with Nova-A standard or the same Tris buffer containing an additional 140 mmol of NaCl per liter. Serial dilutions were made with the appropriate water, Tris-buffered, or Nova-A standard reconstitution solution to provide various protein concentrations at the same Na^+ concentration.

For studies of the influence of pH, the lyophilized sera were reconstituted with water, and the de-ionized and lyophilized sera reconstituted with 140 mmol/L NaCl, 4 mmol/L KCl. The pH of the pooled sera; lyophilized and reconstituted sera; and de-ionized, lyophilized, and reconstituted sera (all at final protein concentrations of 70 g/L) was adjusted with 1.0 mol/L HCl or 1.0 mol/L KOH. The pH of a 140 mmol/L NaCl solution was similarly changed.

Human albumin, human γ-globulin, and bovine albumin (all purchased from Sigma) were de-ionized and lyophilized before use. These proteins were reconstituted in Nova-A standard, filtered, and diluted with Nova-A standard to provide various protein concentrations.

Other procedures. Carbon dioxide was removed from pooled sera by acidifying with HCl to pH ~3.0, followed by bubbling nitrogen through the sera for 2 h at room temperature. Sera were then neutralized with NaOH. To assess the effects of an anion-exchange resin, we mixed some of the pooled sera and also the CO_2-free sera prepared from it with Sephocel resin (Pharmacia), stirring for 2 h, then allowing the resin to settle.

Serum samples from patients at Barnes Hospital were selected for a wide range of protein values. We used only samples with no evidence of a monoclonal protein by cellulose acetate electrophoresis.

Results

We assessed the influence of water content on flame-photometric and direct-potentiometric determinations of Na^+ by using different materials to change the water content. When the water content was altered by the addition of silica, the percentage that the flame-photometric values were of the direct-potentiometric sodium values was identical to the measured water content (Figure 3). Similar results were observed when water content was changed with de-ionized serum (Figure 4) or with human albumin, human γ-globulins, or bovine albumin (correlation coefficient = 0.976, 0.961, 0.954, respectively). Thus, changing the water content by addition of an inert substance (silica), de-ionized sera, or individual proteins changed the percentage of flame-photometric to direct-potentiometric sodium values in a manner that was identical to changes in the water content.

However, the percentage of flame-photometric to direct-potentiometric sodium values was not identical to the water content in 35 patients in whom protein values ranged from 30 to 110 g/L (Figure 5). The percentage of Na^+ values was 99.1 ± 1.9% and the water content was 92.0 ± 1.4%, a discrepancy of 7.1% between the measured percentage of Na^+ values and that predicted by water content.

Bicarbonate having been reported to bind Na^+ (*29*), we removed the CO_2 from pooled sera. This resulted in a decrease in the percentage of flame-photometric to direct-potentiometric Na^+ values by 1.4 ± 0.9% (n = 5). Thus, the major factor responsible for the discrepancy between the percentage of Na^+ values and water content cannot be bicarbonate. However, when the CO_2-free sera was mixed with anion-exchange resin, the percentage of Na^+ values decreased by 5%. Therefore, we assessed the influence of de-ionization.

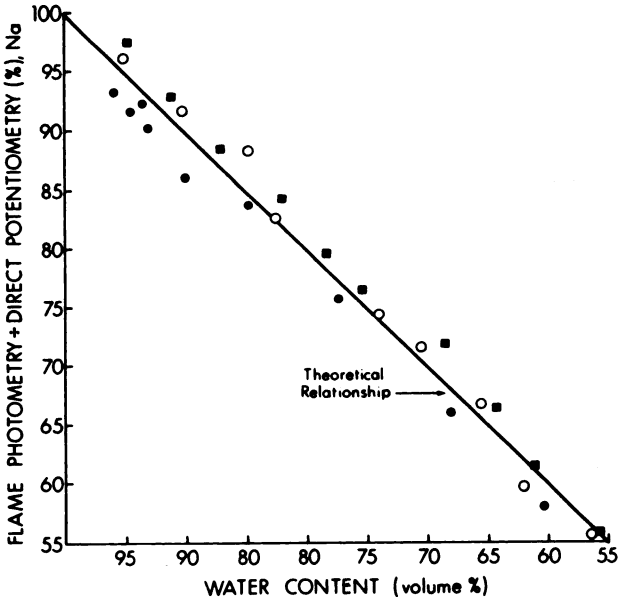

Fig. 3. Relationship between the percentage of flame-photometric to direct-potentiometric measurement of sodium and the measured water content for solutions containing silica

The three symbol sets represent the results of three independent experiments. Percentage of Na^+ values = 1.031 (water content) + 1.7; $r = 0.993$. The *solid line* represents the predicted change in the percentage of Na^+ values as the water content changes

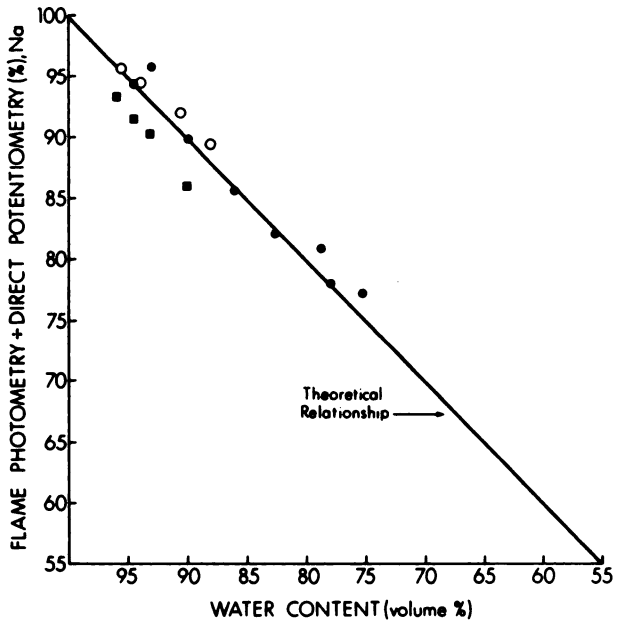

Fig. 4. Relationship between the percentage of flame-photometric to direct-potentiometric measurement of sodium and the measured water content for solutions containing de-ionized human sera

Solid line and symbols as in Fig. 3. Percentage of Na^+ values = 0.975 (water content) + 2.9; $r = 0.986$

We compared the percentage of Na^+ values with the water content for pooled sera; lyophilized and reconstituted sera; and de-ionized, lyophilized, and reconstituted sera. The average percentage of Na^+ values (and the water contents) for five experiments, when we used water to reconstitute the lyophilized sera and Nova-A standard to reconstitute the de-ionized and lyophilized sera, were: pooled sera, 98.0 ± 1.0% (91.4 ± 0.4%); lyophilized sera, 97.8 ± 2.3% (90.6 ± 1.2%); and de-ionized and lyophilized sera, 92.9 ± 2.2% (90.4 ± 1.0%). Thus, the de-ionization process caused some type of change

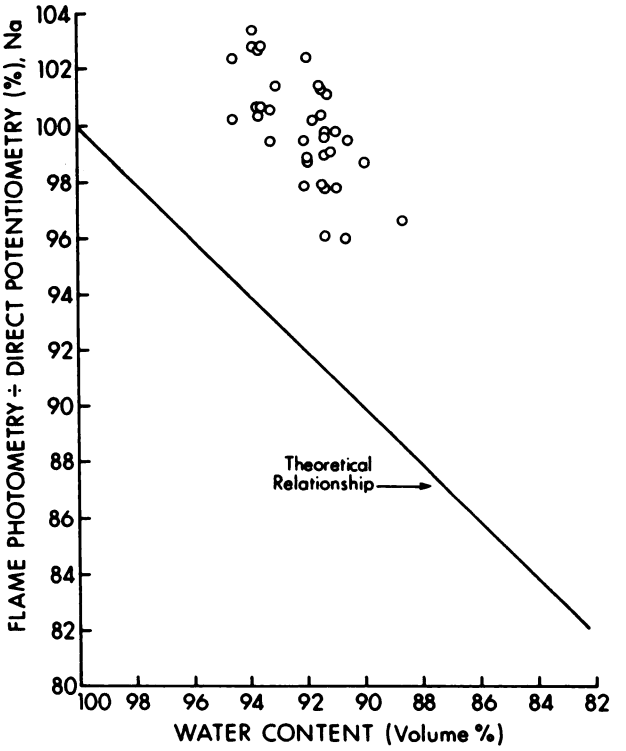

Fig. 5. Relationship between the percentage of flame-photometric to direct-potentiometric measurement of sodium and the measured water content in sera from 35 patients

The range of protein values in these patients was 30 to 110 g/L (mean 71 g/L). The mean water content was 92.0% and the mean percentage of Na^+ values was 99.1%, a discrepancy of 7.1%

in the sera such that the percentage of flame-photometric to direct-potentiometric sodium values approached the water content. This was not related to lyophilization.

When we measured the pH of the above samples, we found that the de-ionized and lyophilized preparations were at pH 5–6 and the other preparations at pH 8–9. To avoid these pH differences, we reconstituted the lyophilized sera and de-ionized and lyophilized preparations with Tris buffer. Under these conditions we obtained a percentage of Na^+ values for the de-ionized and lyophilized preparation that was 1.2% lower than that for the lyophilized preparation, instead of ~5% lower values we obtained for the unbuffered solutions.

We therefore assessed the influence of pH over a wide range in the pooled, lyophilized, and de-ionized and lyophilized preparations. Preliminary data (Figure 6) indicate that the percentage of flame-photometric to direct-potentiometric Na^+ values in pooled sera; lyophilized and reconstituted sera; or de-ionized, lyophilized, and reconstituted sera varies as a function of pH. This effect of pH is not observed in an aqueous NaCl solution. Similar results were obtained when the Ektachem 400 was used for the direct potentiometric measurement of Na^+ (data not shown).

Discussion

The relationship of flame-photometric to direct-potentiometric values for Na^+ should be a function of the effective water content of plasma. Our data for increasing amounts of silica, de-ionized sera, or individual proteins confirmed the relationship between the percentage of Na^+ values and water content (Figures 3 and 4). Other studies from this laboratory have investigated sera with altered water contents from hyperlipemia (*13*) and hyperproteinemia (*17*). Both of these conditions lead to a lower percentage for flame-photometric as compared with direct-potentiometric values for sodium.

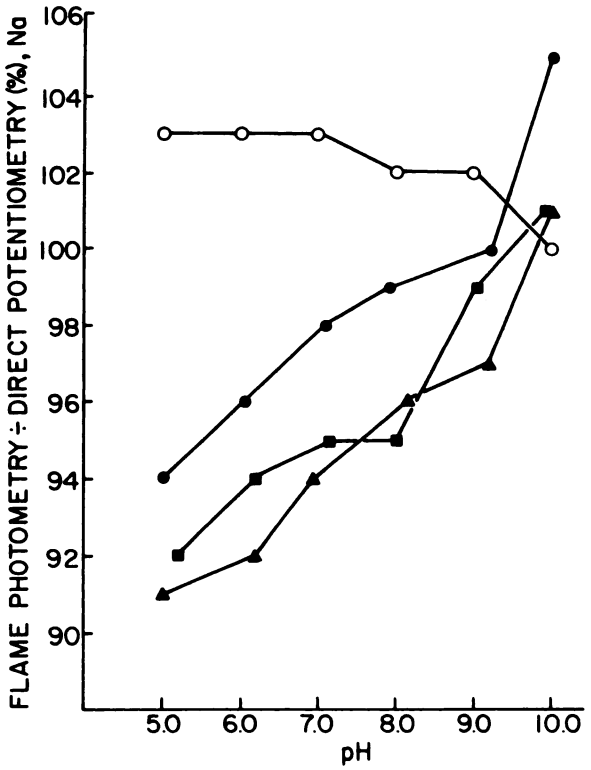

Fig. 6. Relationship between the percentage of the flame-photometric to direct-potentiometric measurement of sodium as a function of pH

O, aqueous 140 mmol/L NaCl control; ●, pooled sera; △, lyophilized sera; ■, de-ionized and lyophilized sera

Table 1. Measured and Predicted Values for Linearity Studies with the Nova-1 [a]

NaCl concn	Predicted value: Based on activity coefficient (γ)	Predicted value: Based on γ and liquid junction potential	Measured value
		mmol/L	
50	55.0	50.5	51.8
100	104.0	102.2	100.8
125	127.5	126.6	124.8
150	150.6	150.8	150.6
175	173.1	174.7	174.8
200	195.4	198.7	199.2

[a] The influence of ionic strength on the activity coefficient was calculated based on the Debye–Hückel equation and compared with that calculated for the Nova-A standard. The liquid junction potential for each solution, relative to 2 mol/L KCl, was calculated from the Henderson equation, compared with that for the Nova-A standard, and converted to sodium values by assuming a nernstian slope.

Under these conditions we found that the direct-potentiometric values agreed best with the osmolality and the patients' clinical condition.

However, our results from patients with a wide range of protein values (Figure 5) indicate that the percentage of Na^+ values in human sera is not equivalent to the measured water content, unlike our findings for the added silica, de-ionized sera, or individual proteins. There was a discrepancy of 7.1% between the percentage of Na^+ values and the water content. Currently, we know of no satisfactory explanation for this discrepancy. The explanations being considered include a calibration error, measurement artifact, Na^+ binding, and water binding.

Calibration error. Czaban and Cormier have suggested (*25*) that the discrepancy between the percentage of the Na^+ values and the water content is caused by a problem in using aqueous NaCl solutions as the calibrant, owing to a mismatch of activity coefficients and residual liquid junction potentials. This appears unlikely because ultrafiltrates of patients' samples give the same Na^+ value by either measurement technique (*20*). In addition, the Nova instrument in which 2 mol/L KCl is used as a salt bridge, and the Eastman instrument in which the salt bridge was a physiological solution of Na^+, K^+, Cl^-, and HCO_3^-, gave similar values for the effects of pH on Na^+ values in sera. Moreover, results obtained for NaCl solutions with a 2 mol/L KCl salt bridge varied linearly with Na^+ concentration, owing to the roughly compensating influence of ionic strength on the activity coefficient and residual liquid junction potential (*31* and Table 1).

Measurement artifact. There are no data supporting a possible measurement artifact in the direct-potentiometric systems. Ultrafiltrates give the same Na^+ value by direct potentiometry and flame photometry. Moreover, increasing concentrations of protein give the same direct potentiometric value (*15*, *20*, *32*, *33*) and produce a percentage of flame-photometric to direct-potentiometric values in agreement with water content.

Sodium binding to bicarbonate has been reported, with an estimated 2.7% of the Na^+ being bound in a 25 mmol/L HCO_3^- solution at physiological ionic strength (*29*). We confirm this apparent binding but find a slightly lower magnitude of 1.9% Na^+ being bound in similar solutions (data not shown). In addition, removal of CO_2 from sera gave a 1.4% lower percentage of Na^+ values. Therefore, bicarbonate binding apparently accounts for an increase of ~2% in the percentage of Na^+ values. This still leaves a discrepancy of ~5% to be accounted for.

Na^+ binding to protein in pooled sera has been considered unlikely, on the basis of recovery and nuclear magnetic resonance data (*30*). Re-examination of Na^+ binding to serum proteins may be necessary, given our findings of a pH influence on the percentage of flame photometric to direct potentiometric Na^+ values. Our experience with de-ionized sera, which we once considered evidence for a lack of Na^+ binding, seems only to reflect the lower pH of our preparations of this material. Older studies with use of collodion membranes and a far narrower pH range reported data with human albumin consistent with little Na^+ binding at neutral pH but Na^+ binding at higher pH values (*34*).

Water binding. The last explanation to be considered in accounting for the discrepancy between the percentage of flame-photometric to direct-potentiometric Na^+ values and the water content is the possibility that the effective water content is greater than the measured water content. It has been reported that water binds to proteins (*35–37*). If 0.5 g of H_2O per gram of serum protein were so associated (*35*), then 3.5 g of water would be bound at a protein value of 70 g/L. Thus, the total water content could be 92% but the effective water content might be 96%. Direct measurements of bound water will be necessary to test this explanation.

We have presented data to clarify the interpretive problem that currently exists when flame-photometric and direct-potentiometric values for Na^+ in blood are compared. The water content clearly influences the flame photometric determination, and in clinical situations with altered water content the direct-potentiometric values appear to be more nearly accurate (*13*, *17*). In normal human sera there is a discrepancy between the percentage of Na^+ values and the water content that cannot be adequately explained at this

time. A system to study this phenomenon, based on the observation that pH alters the percentage of Na^+ values, is being developed, which we hope will allow us to understand more about the nature of sodium partition and its measurement in blood.

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