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THE DETERMINATION OF SODIUM IN BODY FLUIDS BY THE GLASS ELECTRODE*

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The use of glass electrodes for the determination of pH is well established. The possibility of measuring alkali ions in biological fluids by an electrode potential offers several advantages over other physical methods such as flame photometry (2). Since the publication of work by Eisenman, Rudin, and Casby (3) in 1957 demonstrating that the ternary glass system Na_2O-Al_2O_3-SiO_2 may be systematically varied to produce electrodes with high affinity for selected cations, principally sodium and potassium, interest in such glasses has become widespread. These authors found no indication of glass poisoning by constituents of serum, cerebrospinal fluid, or brain homogenate, the electrodes showing expected potentials with known concentration changes of Na^+, H^+, K^+, or Ca^{++}.

Bower (4) found that sodium concentration of irrigation waters and saturation extracts of soils as determined by the electrode agreed closely with those obtained by flame photometry. Friedman and associates have demonstrated the ability of electrodes to follow cyclic changes of sodium concentration in mixed sodium and potassium solutions (5) and in whole blood (6), and Hinke (7) has constructed microelectrodes for the measurement of intracellular sodium and potassium activities.

To date, however, there has been no detailed report on the use of such glasses in biological fluids, or upon the accuracy with which such measurements may be made as a routine clinical or research tool. The purpose of this report is to provide such data for sodium ion by using a sodium aluminum silicate glass. Corroborating data will also be presented for a lithium analog glass. It will be shown that sodium activity (and concentration) may be rapidly determined in urine, serum, cerebrospinal fluid (CSF), whole blood, and plasma with a high degree of accuracy and reproducibility. Electrode response as a function of biological variables such as pH and temperature is the subject of a subsequent report (8).

THEORETICAL CONSIDERATIONS

Since the technique employed embodies the mathematical and electrochemical theories used in the determination of pH, a brief review of the subject is pertinent to the present investigation.

Although the theory of the glass (H^+) electrode has not yet been completely elucidated, its action appears to be related to the transfer of ions through the glass (9). The subject has been extensively reviewed by Dole (10) and more recently by Eisenman (11). The electromotive force (E) obtained with a pH electrode depends upon the difference in hydrogen ion activities across the glass membrane, and may be expressed by (12):

$$E = E^0 + \frac{2.3\,RT}{nF} \log_{10} a_{m^+}, \qquad [1]$$

where E^0 is constant for a given electrode, n is the valency of the ion, and a_{m^+} is the activity of the cation in the test solution.

Since the measured e.m.f. (electromotive force) varies with the logarithm of a_{m^+}, only small changes in e.m.f. are produced by large changes in a_{m^+}. For any univalent cation such as sodium, the expected change in e.m.f. for a

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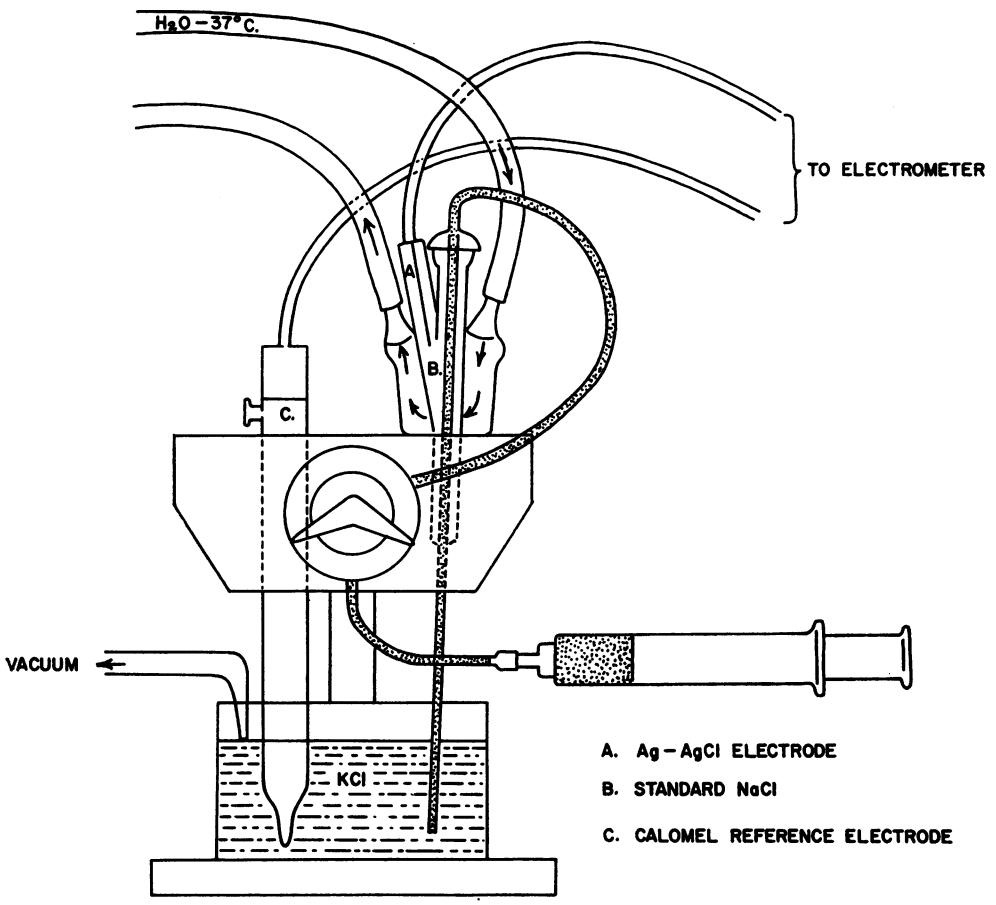

FIG. 1. DIAGRAM OF NAS_{11-18} (RISEMAN) ELECTRODE. Standard or test solution is introduced into capillary electrode (vertical stippled area), which is surrounded by standard 0.05 N NaCl solution (B) containing Ag-AgCl wire lead (A). Constant temperature is maintained by a circulating water enclosure. Excess test solution enters saturated KCl reservoir, the level of which is kept constant by vacuum. A fiber-type calomel half-cell (C) serves as reference.

tenfold change in ion activity would be about 61.7 mv at 37° C (i.e., $2.3\,RT/nF$). Theoretically, therefore, the same change in potential should be registered whether ion activity changes from 0.1 mEq per L to 0.2 mEq per L, or from 100 mEq per L to 200 mEq per L.

Equation 1, which applies to one species of ion in solution, has been elaborated upon by Eisenman and associates (3) to yield a precise description of the glass electrode potential in mixtures of any two univalent cations, represented by the empirical equation:

$$E = E^0 + \frac{2.3\,RT}{nF}\log_{10}\left[(A^+)^{1/n_{AB}} + k_{AB}^{1/n_{AB}}(B^+)^{1/n_{AB}}\right]^{n_{AB}}, \quad [2]$$

where k_{AB} and n_{AB} are empirical constants for a given glass composition and cation pair (A^+ and B^+). With sodium-sensitive glasses in solutions containing $Na^+ - K^+ - H^+$ mixtures, k_{NaK} was found to vary with pH, approaching a constant value at low hydrogen ion concentration. This latter coefficient, k_{AB}, may be thought of as an expression of the "relative sensitivity" of a particular glass electrode for a given pair of ions (3), i.e., how well the electrode "sees" one cation in preference to another. The value of k_{AB} may be conveniently determined by taking e.m.f. measurements at two different concentrations of one ion in a constant concentration of the other and solving the simultaneous equations. Thus, a value of k_{NaK} of 0.01 signifies that the electrode "sees" sodium ion (A^+) one hundred times as well as potassium (B^+), or that 0.01 M Na^+ would register a potential equal to that produced by 1.0 M K^+.

With the glass electrode, it is the activity (a) of an ion which is measured, representing the product of an appropriate activity coefficient (γ) and ionic concentration (13):

$$a_{Na^+} = C_{Na} \cdot \gamma_{Na^+}. \qquad [3]$$

Since the activity coefficient cannot be determined for a single ion species, γ_{Na^+} refers to the mean coefficient, which is dependent upon the accompanying anion in the solution. In very dilute solutions it is generally satisfactory to assume that the activity of an ion is equal to its concentration, i.e., $\gamma = 1$. In more concentrated solutions, activity coefficients are related to the ionic strength of the solution (14). At ionic strengths near 0.01 M, the activity coefficient may be calculated by a refined form of the Debye-Hückel equation (15). In order to express electrode data obtained from biological fluids in terms of concentration, it is necessary to know the appropriate activity coefficient. This, in turn, depends upon the ionic strength of the solution and the temperature at which the determination is made.

MATERIALS AND METHODS

Samples of urine, serum, whole blood, and plasma were obtained from normal hospital personnel. Urine, serum, and CSF were obtained from hospitalized patients with a variety of diseases. The CSF samples had been collected over a period of several months and stored at −10° C prior to analysis. Otherwise, all analyses were performed on the day of sample collection.

The determination of sodium activity was made with a capillary electrode composed of Eisenman's NAS_{11-18} glass[1] (Na_2O, 11 moles per 100 ml; Al_2O_3, 18 moles per 100 ml; SiO_2, 71 moles per 100 ml), using a KCl salt bridge with a Metrohm fiber-type calomel half-cell as the reference electrode. A diagram of this electrode set-up, referred to as the "Riseman electrode," is shown in Figure 1 and is essentially a modification of a blood pH electrode. The NAS_{11-18} glass was a capillary tube (o.d., 1.0 to 1.5 mm; i.d., 0.5 to 1.0 mm; wall thickness about 0.25 mm) surrounded by a solution of 0.05 N NaCl containing a central Ag-AgCl lead. The volume of unknown or standard solution required for analysis was less than 0.5 ml, and no dilution of the sample was necessary.

For comparison, sodium activity was also determined in 36 urines by means of an immersion (Haber bulb) electrode composed of lithium aluminum silicate glass of unknown composition, obtained from Beckman Instruments Co. (experimental electrode no. 78178V). A Beckman fiber-type calomel half-cell was used as reference. Both electrodes were immersed in a beaker containing the sample, surrounded by a constant temperature water bath. The volume of sample thus required was about 20 ml, and mixing was achieved by a magnetic stirrer.

All potentiometric measurements were made with a Riseman Development Laboratory pH-meter, model MV-1. This is a null balance, chopper amplifier meter with separate inputs for two different pH or cation-sensitive electrodes, and can be read directly on a vernier dial to 0.001 pH unit or ±0.1 mv. Continuous readings over a range of 10 pH units or 1.0 volt may be achieved. Instrument drift is less than 50 μv per hour, noncumulative. All determinations of sodium activity were made at 37° C with a Haake model F constant temperature water bath. This temperature was selected so that activity coefficients thus derived could be applied by others in *in vivo* experiments with glass electrodes.

Calibration of the electrodes was made with 0.010 N, 0.050 N, and 0.100 N NaCl standards with assumed sodium activities at 37° C of 9.0 mEq per L, 40.7 mEq per L, and 77.0 mEq per L, respectively. These latter values were calculated from the Debye-Hückel expression (15). These same standards were also used each day for calibration of the flame photometer.

Although the output signal from the electrometer may be readily amplified and recorded on a moving chart, recalibration necessitated by slight drifts in standard curves makes the calculations somewhat tedious and time consuming. To facilitate the direct calculation of activities, we have constructed a "cation activity board." A large sheet of semilog graph paper is mounted on a plywood board. Sliding over this is a Plexiglas transparent rule with a finely etched line on the under surface; the slope of the rule is adjustable. Millivolt potential (abscissa) is plotted against ionic activity and the slope of the standard curve adjusted. This slope is remarkably constant during the course of a day, but may vary as much as 1.5 mv from day to day. Although the slope of the standard curve is constant for any given day, the entire curve may shift several millivolts. For this reason, the potential of a standard NaCl solution (0.050 N) has been measured between each determination of activity in the unknown sample. Such shifts in the standard curve are easily adjusted by sliding the Plexiglas rule so that the millivolt reading of the standard corresponds to the activity of the standard, in this case, 40.7 mEq per L. Sodium activity in the unknown is then read directly from the graph. The entire operation requires only a few seconds, and the need for repeated drawings of standard curves is completely eliminated.

Regarding the properties of the NAS_{11-18} glass electrode, our data concerning the selectivity constant k_{AB} are in agreement with those of Eisenman and associates (3), in that there is somewhat greater selectivity for sodium over potassium at high pH. The selectivity of our particular glass is approximately 100/1 at pH 7 ($k_{NaK} = 0.01$). Although k_{AB} appears to vary also with temperature and preferred ion concentration (8), this is probably of no great significance for sodium determinations in biological

[1] Supplied by Riseman Development Laboratory, Cambridge, Mass.

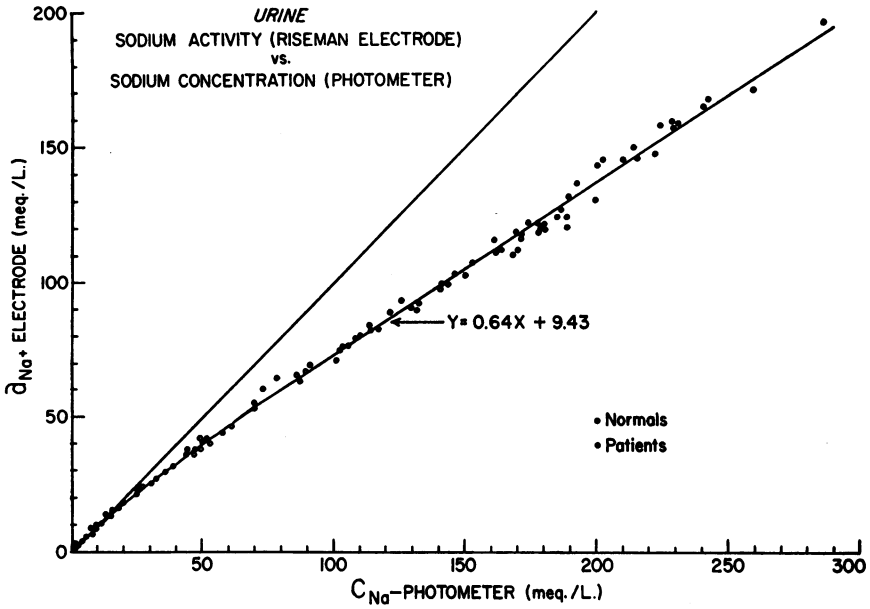

FIG. 2. COMPARISON OF SODIUM ACTIVITIES (NAS_{11-18} ELECTRODE) WITH SIMULTANEOUSLY OBTAINED CONCENTRATION (FLAME PHOTOMETER) VALUES IN 104 URINES Deviation of activity measurements from the 1:1 slope line is evident at concentrations above 15 mEq per L, with re-establishment of linearity at concentrations above 70 mEq per L.

fluids. The NAS_{11-18} glass is 15 to 20 times more sensitive to hydrogen ion than to sodium ion, but no appreciable hydrogen error is present with sodium concentrations encountered over the physiologic pH range. Even in urines at pH 4.5, the "hydrogen error" would be less than 1 mEq per L. These same considerations also apply to the Beckman lithium analog glass used in some of the experiments, although the selectivity of this glass for Na^+ over K^+ is somewhat greater than that of the NAS_{11-18} composition ($k_{NaK} < 0.01$). Neither the NAS_{11-18} nor the Beckman lithium glass showed evidence of anion sensitivity.

All determinations of sodium activity were compared with sodium concentration levels obtained simultaneously by means of a Baird Associates model DB-4 flame photometer. Both the electrode and photometer measurements were made in duplicate, and most of the photometry was performed by one technician. The average difference between duplicate determinations of sodium concentration with the flame photometer for urine, CSF, and serum was 0.8 mEq per L, and the average difference between duplicate activity measurements with the electrode was 0.3 mEq per L. A more precise estimate of replicate variability was calculated by analysis of variance (Table I). The indicated values (2 SD) represent the 95% confidence limit for the true value of any given individual sample. Thus, for a single photometer value of 100.0 mEq per L in urine, the true value would, with 95% confidence, be between 97.66 mEq per L and 102.34 mEq per L. The variability between duplicate determinations with the electrode was about one-half that with the photometer, with serum showing the least variability by both methods.

In order to conserve space, individual subject data, except for whole blood and plasma, are not included in tabular form in the present paper, but are available from the authors upon request.

TABLE I

Variability of replicate sodium determinations

	Photometer (concentration)			Electrode (activity)		
	Urine (42)	Serum (34)	CSF* (34)	Urine (42)	Serum (34)	CSF (34)
Mean	109.8	141.5	141.7	77.6	109.8	105.4
2 SD	2.34	2.07	2.25	0.87	0.42	0.96

* Cerebrospinal fluid.

RESULTS

I. *Urine*

A. *Group I.* Sodium activity was determined in 64 urines from 54 normal subjects and in 40 urines from hospitalized patients. Sodium concentration, as determined by flame photometry, ranged from 26.8 mEq per L to 286.5 mEq per L in the normal group, and from 0.1 mEq per L to 193.0 mEq per L in urines from patients.

When sodium activity (a_{Na^+}) was plotted against the simultaneously obtained flame concentration (C_{Na_P}), a linear function $Y = 0.64X + 9.43$ was obtained for urines with sodium

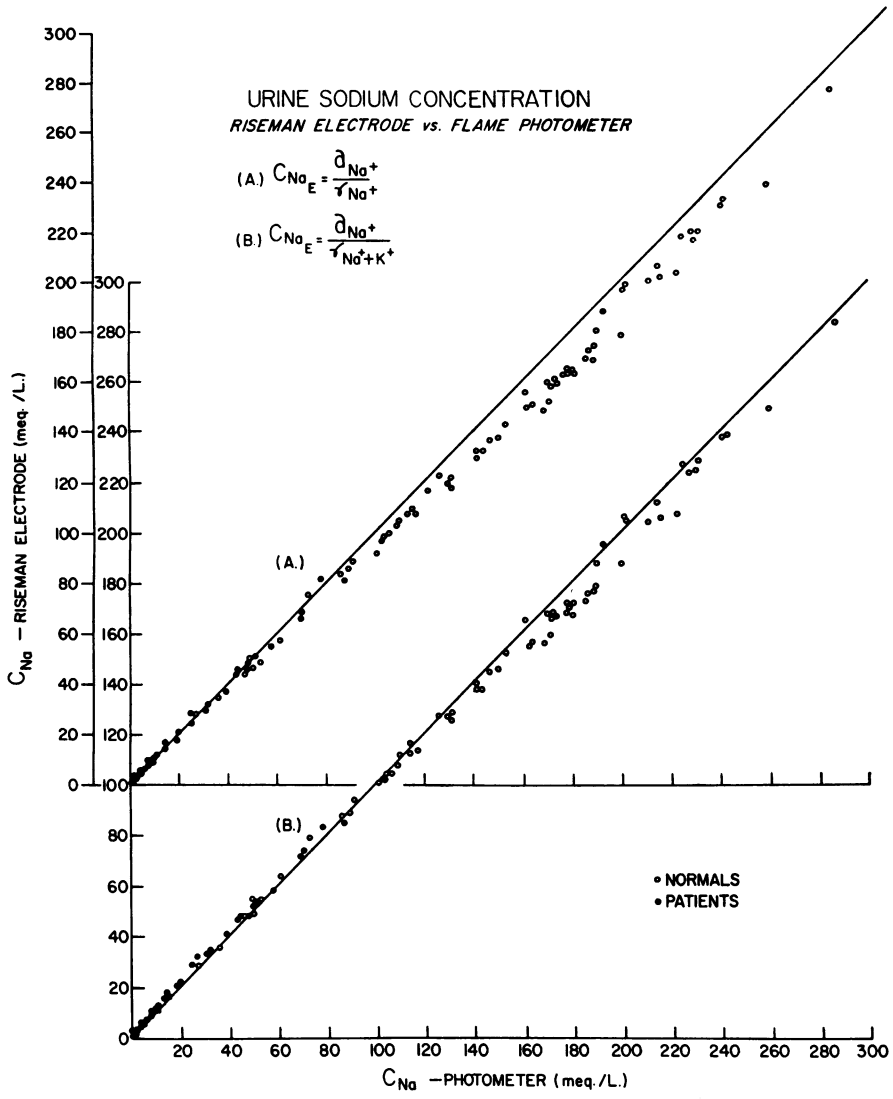

FIG. 3. COMPARISON OF SODIUM CONCENTRATION VALUES IN 104 URINES AS OBTAINED BY FLAME PHOTOMETRY AND THE NAS_{11-18} ELECTRODE. A. Electrode concentrations (C_{Na_E}) have been calculated from activity coefficients for NaCl in aqueous solution according to the relationship: $C_{Na_E} = a_{Na^+}/\gamma_{Na^+}$. B. Electrode concentrations have been calculated from activity coefficients for mixed NaCl–KCl solutions according to the relationship: $C_{Na_E} = \gamma_{Na^+ + K^+}/a_{Na^+}$.

concentrations above about 15 mEq per L (Figure 2). In urines containing 6 to 15 mEq per L, the measured activity was essentially equal to concentration. At sodium concentrations below 6 mEq per L, the measured activity was usually greater than the corresponding concentration value, undoubtedly related to potassium error in the glass. (Thus, for a glass with $k_{NaK} = 0.01$, a urine containing 1 mEq per L sodium and 100 mEq per L potassium would register a potential equivalent to 2 mEq per L sodium.)

There appeared to be no intrinsic difference between urines from normal subjects and those from patients; the function $Y = 0.64X + 9.43$ describes activity-concentration relationships equally well in both. Also, there was no apparent difference in the behavior of urines with high sodium concentrations and those with low levels. When high-sodium urines were diluted 1:1, the

resulting activities were also accurately represented by the above function.

Protein was present in 12 of the patient urines above, and in 5 of these the proteinuria was marked. Sodium activity in each of these showed no appreciable deviation from the mean activity curve for the group as a whole. The effect of various normal urinary constitutents on sodium activity was also studied. The addition of large amounts of urea or uric acid had no appreciable effect on measured sodium activity. Glucose, when added to urine in concentrations ranging from 2% to 6%, was found to increase activity measurements somewhat, the average Δa_{Na^+} being only about 2 mEq per L with 6% glucose.

No attempt was made to relate activity measurements to the pH of urine, since the large number of sample determinations should result in a random pH distribution over any given sodium concentration interval. Also, if urinary sodium activity could be determined without regard to pH, the usefulness of the electrode would be considerably enhanced.

In order to express electrode activity data in terms of sodium concentration, it was necessary to derive the proper activity coefficients. Activity coefficients (γ_{Na^+}) for NaCl in aqueous solution at 37° C were used in an attempt to effect this conversion. These coefficients were calculated according to the Debye-Hückel equation (15) for sodium concentrations up to 100 mEq per L. Above this level, γ_{Na^+} was extrapolated by a line parallel to standard table values (16) for NaCl at 25° C. As may be seen in Figure 3A, sodium concentration values thus obtained by the electrode (C_{NaE}) were less than the corresponding photometric values (C_{NaP}), particularly with concentrations above 50 mEq per L, indicating that sodium activity coefficients for urine are less than those of aqueous NaCl solutions. The average deviation from photometric values in 64 normal urines amounted to −9.7 mEq per L, representing a mean error of −6.0%. In the group of 40 patient urines, with considerably lower sodium concentrations, the mean deviation, without regard to sign, was only 1.3 mEq per L. Clearly, activity coefficients for aqueous NaCl solutions could not be applied to high-sodium urines if great precision were required, but would be adequate if only a rough approximation of sodium concentration were needed, or if the sodium concentration were low.

In order to determine the ion primarily responsible for this suppression of sodium activity, the effect of potassium was investigated. Since activity coefficients for KCl in aqueous solution are somewhat less than those for NaCl (16), the question arises: To what degree does a given amount of potassium suppress sodium activity? If one assumes that suppression by potassium, with the same charge as sodium but with smaller hydrated ion diameter (17), is identical to that produced by an equivalent amount of NaCl (allowing for the difference in activity of these ions in pure solution), calculations may be made from interpolation of standard activity coefficient curves. Thus, if a given urine contained 100 mEq per L Na^+, the presence of 100 mEq per L K^+, as determined by photometry, would lower the mean activity coefficient from 0.770 to 0.722 (disregarding the effects of H^+, NH_4^+, Ca^{++}, etc.). When such activity coefficients were derived for the entire group of urines, sodium concentrations were obtained which agreed more closely with photometric values (Figure 3B). The mean deviation ($C_{NaE} - C_{NaP}$) for the group of 64 normal urines, without regard to sign, was 5.2 mEq per L, or about 3.4%. Thus, as might be expected, the suppression of sodium activity in urine, relative to the activity of aqueous NaCl solutions of equal ionic strength, was primarily related to the presence of potassium, the other major cation in urine. Of course, the derived coefficient $\gamma_{Na^+ + K^+}$ was of no practical value in interpreting electrode activity data, since a foreknowledge of both sodium and potassium concentrations was required.

An empirical relationship between measured sodium activity and the activity coefficient necessary to achieve correspondency to photometric values was therefore derived for each urine. This empirical coefficient, γ_{urine}, was calculated by dividing measured activity by the sodium concentration as determined by photometry: $\gamma_{urine} = a_{Na^+}/C_{NaP}$. A table of mean activity coefficients was then constructed from the resulting mean curve. These coefficients, γ_{urine}, were then used to calculate the urinary sodium

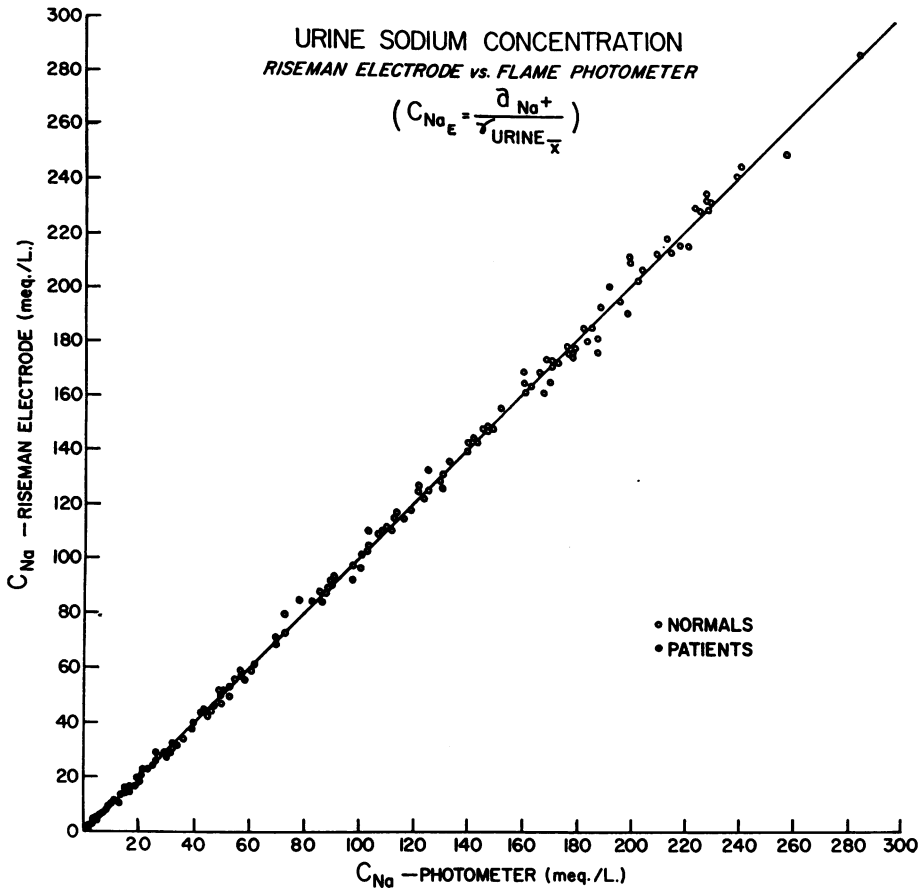

FIG. 4. COMPARISON OF SODIUM CONCENTRATION VALUES IN 156 URINES AS OBTAINED BY FLAME PHOTOMETRY AND THE NAS_{11-18} ELECTRODE. Electrode concentration values have been calculated from the empirical mean activity coefficient curve according to the relationship: $C_{NaE} = a_{NaE}/\gamma_{urine\bar{x}}$.

concentration (C_{NaE}) from electrode activity measurements, according to the relationship: $C_{NaE} = a_{Na^+}/\gamma_{urine\bar{x}}$. The resulting close correlation of these electrode concentrations to the corresponding photometric values is shown in Figure 4. Mean deviation of C_{NaE} from C_{Nap}, without regard to sign, was 3.4 mEq per L, or 2.5% in the 64 normal urines, and 1.1 mEq per L in the forty patient urines. Over-all difference for the 104 urines was 2.6 mEq per L.

B. *Group II.* The usefulness and accuracy of the derived empirical activity coefficients were further tested in an additional 52 urines, including 29 urines from normal subjects and 23 urines from hospitalized patients. The average deviation of the electrode values in these urines from those obtained by photometry was only 2.2 mEq per L, or 2.0%, in the normal subjects and 0.9 mEq per L in the urines from patients. All of these are also plotted in Figure 4. The mean deviation from photometric values for all group I and group II urines (156 samples) was 2.2 mEq per L. In the 93 normal urines, the average deviation of electrode concentrations from photometric values was 3.1 mEq per L, or 2.3%.

C. *Beckman electrode.* In order to determine variability in measured sodium activity between two different glasses, activity measurements were also made in 36 of the above urines by means of a lithium aluminum silicate (Beckman) electrode (Figure 5). Activity measurements with the Beckman electrode were usually slightly lower than those obtained with the NAS_{11-18} glass, but the correlation was quite close ($r = 0.998$). The relation between the two electrode activities could be expressed by the function $Y = 0.97X - 0.36$. When activity coefficients for the

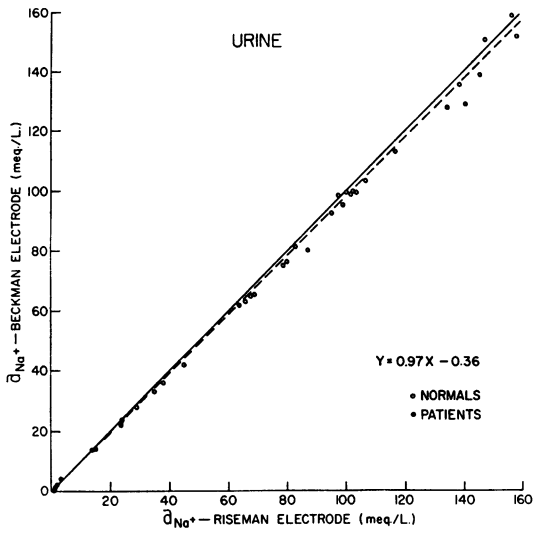

FIG. 5. COMPARISON OF SODIUM ACTIVITIES SIMULTANEOUSLY OBTAINED FROM TWO GLASS ELECTRODES AT 37° C.

NAS_{11-18} glass were used to convert "Beckman activities" to concentrations, the values thus obtained were low when compared with the photometer. An empirical activity coefficient curve was therefore derived for the Beckman electrode as described above for the NAS_{11-18} glass, yielding the hyperbola $Y = 0.646 + (3.25/X)$. When the activity coefficients obtained from this function were used to convert activities to concentrations, mean deviation from the photometric values was 3.3 mEq per L, compared with an average error of 1.8 mEq per L in these same urines with the NAS_{11-18} glass. The somewhat greater accuracy with the NAS_{11-18} electrode may, in part, reflect the fact that the activity coefficient curve for this glass had been determined by a larger number of observations and, therefore, with greater certainty.

II. *Serum*

Sodium activity was determined with the NAS_{11-18} electrode in a total of 104 sera, including sera from 23 normal subjects and 20 individual patients, and 61 pooled sera from patients. The resulting plot of activities against the corresponding photometric values, represented by the linear function $Y = 0.748X + 4.11$ ($r = 0.957$), is shown in Figure 6. The mean activity coefficient for the 23 normal subjects was 0.780 ± 0.001,[2] which was not significantly different from the mean of 0.774 ± 0.002 in the 20 individual patients or from the mean of 0.779 ± 0.001 in the 61 pooled sera. The somewhat greater variability in activity coefficients among the latter 2 groups might be expected, since a more extreme concentration range was encountered in these sera.

The appropriate mean activity coefficients ($\gamma_{\text{serum}_{\bar{x}}}$), derived from the linear function above, were then used to convert activities to concentrations according to the relationship: $C_{Na_{\bar{x}}} = a_{Na^+}/\gamma_{\text{serum}_{\bar{x}}}$. The mean deviation of the concentration values thus obtained from the corresponding photometric values, without regard to sign, was 1.3 mEq per L in the entire group, representing an average error of 0.9%.

It should be noted that sodium activities in sera were somewhat higher than those which would be expected in pure aqueous solutions of NaCl at 37° C. This discrepancy appeared to be related to the fact that the nonaqueous phase of serum was neglected in the expression of sodium concentration as determined by photometry. When photometric values were expressed in terms of sodium concentration in serum water, resulting serum activity coefficients were somewhat less than those for corresponding aqueous NaCl solutions. These coeffi-

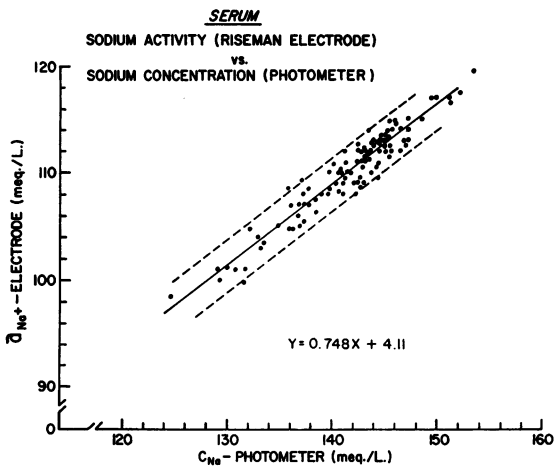

FIG. 6. THE RELATIONSHIP OF SODIUM ACTIVITIES IN 104 SERA, AS DETERMINED WITH THE NAS_{11-18} ELECTRODE, TO SIMULTANEOUSLY OBTAINED CONCENTRATION (PHOTOMETER) VALUES. The solid line is the group mean; the dotted lines represent the 95% confidence limit (±2 SD).

[2] 1 standard error of the mean.

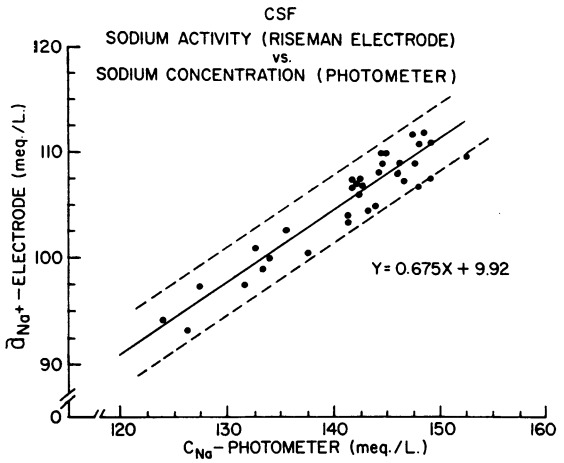

FIG. 7. THE RELATIONSHIP OF SODIUM ACTIVITIES IN CEREBROSPINAL FLUID, AS DETERMINED WITH THE NAS_{11-18} ELECTRODE, TO SIMULTANEOUSLY OBTAINED CONCENTRATION (PHOTOMETER) VALUES. The solid line is the group mean; the dotted lines represent the 95% confidence limit (±2 SD).

cients correlated very closely when the average serum water content was assumed to be 96% by volume (0.747 for serum vs 0.750 for NaCl at a sodium concentration of 140 mEq per L).

III. *Cerebrospinal fluid*

The NAS_{11-18} electrode was used to determine sodium activity in the CSF of 26 patients and in 8 pooled samples from other patients. The resulting plot of activities against simultaneously obtained photometric concentrations is shown in Figure 7 and is represented by the function $Y = 0.675X + 9.92$. Mean activity coefficients for CSF were quite close to those for pure NaCl solutions (0.745 for CSF vs 0.750 for NaCl at 140 mEq per L sodium concentration), and the mean activity coefficient for the entire group of CSF determinations was 0.744 ± 0.002. With mean coefficients ($\gamma_{CSF_{\bar{x}}}$) from the above linear function, the average deviation of electrode concentration values from corresponding photometric values, without regard to sign, was 2.2 mEq per L.

IV. *Whole blood and plasma*

In order to determine the effect of whole blood on electrode potential, activity measurements were made in samples of fresh blood from nine normal subjects and compared with the activities simultaneously obtained in the plasma from these same specimens (Table II). Heparin, containing about 440 mEq per L sodium, was used as anticoagulant (0.2 ml heparin in 20 ml blood), and accounts for the high sodium levels encountered in the analyses.

Whole blood activities were quite close to those obtained from the corresponding plasmas, with a mean difference, without regard to sign, of 0.9 mEq per L. Accordingly, there was no significant difference in mean activity coefficients of 0.757 ± 0.003 and 0.754 ± 0.003 in whole blood and plasma, respectively, uncorrected for nonaqueous phases.

DISCUSSION

The subject of cation-selective glass electrodes and their mode of operation has recently been extensively reviewed by Eisenman (11). From the present work, a few comments seem warranted in regard to the use of such electrodes in biological systems.

The outstanding advantage of electrodes over other physical methods of measurement lies in the fact that the activity of these ions may be directly determined. There is little doubt that ionic activity has greater physiologic significance than ionic concentration, and it seems inevitable that medical research will become more and more oriented towards activity, particularly as more specific cation-selective glasses become available. This seems especially true for di-

TABLE II

Whole blood and plasma sodium activities from nine normal subjects

Subject	C_{Na_P}*	Whole blood		Plasma	
		a_{Na^+}	γ_{Blood}	a_{Na^+}	γ_{Plasma}
	mEq/L	*mEq/L*		*mEq/L*	
D.A.	158.6	118.0	0.744	119.5	0.754
R.W.	151.3	115.5	0.763	114.5	0.757
R.S.	159.0	120.5	0.758	119.8	0.753
J.R.	153.8	118.2	0.768	117.5	0.764
M.S.	157.3	118.0	0.750	117.8	0.749
F.D.	156.0	118.5	0.760	118.8	0.762
H.A.	154.0	117.8	0.765	117.5	0.763
R.L.	160.0	120.0	0.750	118.5	0.741
D.W.	161.6	122.5	0.758	120.5	0.746
Mean	156.8	118.8	0.757	118.3	0.754
SE		0.66	0.003	0.59	0.003

* Sodium concentration (photometer).

valent ions, most notably Ca^{++} and Mg^{++}, where the ionized fraction is recognized as the physiologically active species. In this regard, it is often convenient to express electrode data in terms of the negative logarithm of the activity, as in pH measurements. Thus, a sodium or potassium activity of 1.0 mEq per L (0.001 N) would be represented by $pNa^+ = 3.0$ or $pK^+ = 3.0$, respectively. In conjunction with Eisenman (18), we suggest that + superscripts be used for such notations in order to avoid confusion with the more commonly used pK, representing the negative logarithm of a dissociation constant.

Other advantages of glass electrodes relate to their ease of handling and operation, reproducibility, and rapidity of response. Friedman and associates (5, 6) have established their usefulness in the continuous *in situ* or *in vivo* monitoring of ionic activity, and Hinke (7) has developed electrodes for the direct measurement of intracellular ionic activity. It should be emphasized that, in the present work, sodium activity has been directly determined in urine and other extracellular fluids without the necessity of dilution or other alteration of the specimen. Equilibrium potentials are usually obtained within 3 minutes, although in urines with very low sodium activity (<1 mEq per L) an additional 3 to 5 minutes may be required for great accuracy.

It has been shown that the activity response of two different sodium-sensitive electrodes in body fluids is linearly related to sodium concentration as determined by flame photometry. When expressing electrode data in terms of concentration instead of activity, the fundamental problem is, therefore, the derivation of appropriate activity coefficients for each fluid under investigation. This has been done for NAS_{11-18} glass in urine, serum, CSF, whole blood, and plasma, and also for a lithium aluminum silicate glass in urine. Although the correlation between activity measurements with two separate glasses is quite close, it seems advisable, for very precise work, to construct empirical activity coefficient curves for any given glass composition and for any given fluid.

Activity coefficients for CSF, whole blood, and plasma do not vary greatly from the corresponding coefficients for NaCl in pure aqueous solution. The latter coefficients may, therefore, be useful for the rough approximation of sodium concentration in these fluids. These findings would also seem to indicate that the effect of erythrocytes and protein on electrode response is negligible. When corrections were made for the nonaqueous phase of serum, activity coefficients also corresponded closely with those for pure NaCl solutions. The serum data must be interpreted with caution, however, since a rather narrow concentration range was encountered in the present study. For urine, it has been shown that the depression of sodium activity below that for corresponding aqueous solutions of NaCl is due primarily to the presence of potassium ion. Glucose in high concentration tended to increase sodium activity slightly, while urea and uric acid appeared to have little or no effect. Again, the use of pure NaCl activity coefficients would be useful if only a rough approximation of sodium concentration were required.

Although the average variability between electrode activity measurements and flame concentration measurements is not great, in some urines it appears to be real. Undoubtedly, part of this variability is related to hydrogen ion effects and to errors in measurement for both the electrode and photometer. In an occasional urine, however, the measured sodium activity may be consistently higher or lower than that expected from the mean derived curve. This probably represents "biologic variability" in the sense that the total ionic strength of two urines may vary considerably even though their sodium concentrations are identical. Although the difference is usually small, this possible source of error should be considered in situations where very precise work is required.

It is clear that glass electrodes of the sensitivity used in the present study are quite adequate for work with biological fluids. The usefulness of such electrodes will undoubtedly increase as other specific cation-sensitive glasses become available. In addition, it may be possible to develop anion-sensitive glasses. Work is currently in progress with a potassium-selective glass which is also quite sensitive to ammonium ion. Preliminary data indicate that

the measurement of urinary potassium is feasible with this technique, and it may be possible to determine urinary ammonia as a by-product. The selectivity of this particular glass for potassium is too low for accurate determination in serum, but this problem should be solved when more selective glasses become available.

We believe that electrodes may find considerable usefulness as a routine clinical or research tool. This might readily be accomplished by the construction of a battery of electrodes, including those selective for H^+, K^+, Na^+, and Ca^{++}. By the introduction of a sample into each electrode simultaneously, with a common reference, the time required for equilibration would be no greater than that for one of the electrodes. The resulting activities could easily be monitored through appropriate recording devices, with the achievement of virtual automation.

Finally, perhaps the greatest usefulness of such electrodes will be in the determination of divalent ionic activity, particularly Ca^{++} and Mg^{++}. As noted by Eisenman (11), the monovalent-sensitive glasses (such as the NAS_{11-18} elec trode) respond to doubly charged cations with the expected slope of $RT/2F$ in pure solutions, but their sensitivity for such ions is so low that presently available glasses are of little use in the determination of divalent ions in biological materials. Recently, however, Garrels, Sato, Thompson, and Truesdell (19) have discovered the existence of a class of glasses having important sensitivity to doubly charged cations such as Ca^{++}. Thus far, all of the glasses studied in this laboratory which have shown reasonable calcium sensitivity have also been quite sensitive to ions in the monovalent series. The determination of calcium activity in biological materials may, therefore, require very accurate measurement of sodium and potassium, and perhaps hydrogen and ammonium activities.

SUMMARY

Until recently, there has been no method for the direct determination of activity of alkali ions in biological fluids, physiological data having been expressed solely in terms of concentration. In the present paper, the mathematical and electrochemical techniques for the determination of pH have been applied to the potentiometric determination of sodium ion in body fluids by selective glass electrodes. The method is rapid, highly reproducible, and requires no dilution or alteration of the sample. Sodium activity in urine, serum, cerebrospinal fluid, whole blood, and plasma was linearly related to sodium concentration as determined by flame photometry. Electrode data could also be expressed in terms of concentration by derivation of appropriate empirical activity coefficients for each fluid. Results agreed with flame photometer values within about 2%. Protein, erythrocytes, urea, and uric acid had no apparent adverse effect on electrode potential.

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REFERENCES

1. Moore, E. W., and D. W. Wilson. The determination of sodium in body fluids by means of the glass electrode (abstract). J. clin. Invest. 1962, **41**, 1385.
2. Isard, J. O. Alkali ion determinations by means of glass electrodes. Nature (Lond.) 1959, **184**, 1616.
3. Eisenman, G., D. O. Rudin, and J. U. Casby. Glass electrode for measuring sodium ion. Science 1957, **126**, 831.
4. Bower, C. A. Determination of sodium in saline solutions with a glass electrode. Soil Sci. Amer. Proc. 1959, **23**, 29.
5. Friedman, S. M., J. D. Jamieson, M. Nakashima, and C. L. Friedman. Sodium- and potassium-sensitive glass electrodes for biological use. Science 1959, **130**, 1252.
6. Friedman, S. M., J. D. Jamieson, J. A. M. Hinke, and C. L. Friedman. Use of glass electrode for measuring sodium in biological systems. Proc. Soc. exp. Biol. (N. Y.) 1958, **99**, 727.
7. Hinke, J. A. M. Glass micro-electrodes for measuring intracellular activties of sodium and potassium. Nature (Lond.) 1959, **184**, 1257.
8. Wall, R. A., and E. W. Moore. Glass electrode response as a function of temperature, pH and ionic composition. In preparation.
9. Glasstone, S., and D. Lewis. Elements of Physical Chemistry. Princeton, D. Van Nostrand, 1960, p. 551.
10. Dole, M. The Glass Electrode, New York, John Wiley & Sons, 1941.
11. Eisenman, G. Cation selective glass electrodes and their mode of operation. Biophys. J. 1962, part 2, **2**, 259.

12. Leonard, J. E. Glass electrodes for the direct measurement of sodium ion activity in aqueous solutions. Beckman Instruments Co. Reprint No. R-6148, April 1960.
13. Gold, V. pH Measurements. London, Methuen, 1956.
14. Klotz, I. M. Chemical Thermodynamics. Englewood Cliffs, N. J., Prentice-Hall, 1960, p. 310.
15. *Ibid.* p. 329.
16. Handbook of Chemistry and Physics, 42nd ed., C. D. Hodgman, Ed. Cleveland, Chemical Rubber Publishing Co., 1960, p. 2611.
17. Klotz, I. M. Chemical Thermodynamics. Englewood Cliffs, N. J., Prentice-Hall, 1960, p. 331.
18. Eisenman, G. Personal communication.
19. Garrels, R. M., M. Sato, M. E. Thompson, and A. H. Truesdell. Glass electrodes sensitive to divalent cations. Science 1962, 135, 1045.

ANNOUNCEMENT OF MEETINGS

THE AMERICAN FEDERATION FOR CLINICAL RESEARCH will hold its Twentieth Annual Meeting in Atlantic City, N. J., at the Casino Theatre on the Steel Pier on Sunday, April 28, 1963, at 9:00 a.m. Joint sectional meetings with The American Society for Clinical Investigation will be held on Sunday afternoon at Chalfonte-Haddon Hall, and additional meetings sponsored by The American Federation for Clinical Research will be held there on Sunday evening.

THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, INC., will hold its Fifty-fifth Annual Meeting in Atlantic City, N. J., on Monday, April 29, at 9:00 a.m., at the Casino Theatre on the Steel Pier and in simultaneous programs sponsored with The American Federation for Clinical Research on Sunday afternoon, April 28, in Chalfonte-Haddon Hall.

THE ASSOCIATION OF AMERICAN PHYSICIANS will hold its Seventy-sixth Annual Meeting in Atlantic City, N. J., at the Casino Theatre on the Steel Pier on Tuesday, April 30, at 9:30 a.m., and in the Vernon Room, Chalfonte-Haddon Hall, on Wednesday, May 1, at 9:30 a.m.

THE AMERICAN SOCIETY FOR CLINICAL NUTRITION will hold its Third Annual Meeting in Atlantic City, N. J., at the Colton Manor Hotel on Saturday, April 27, from 1:00 to 5:00 p.m.