Factors That Limit Brain Volume Changes in Response to Acute and Sustained Hyper- and Hyponatremia

MALCOLM A. HOLLIDAY, M. N. KALAYCI, and JEAN HARRAH

From the Department of Pediatrics, University of California–San Francisco Medical Center, San Francisco, California 94122 and the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15200

ABSTRACT Rats were made acutely hyper- or hyponatremic by infusion of hypertonic saline or water, respectively. Other rats were maintained in these states from 1 to 7 days to observe the effects of time. Brain tissue water, Na, Cl, and K were compared with serum Na and Cl concentration (Na_E and Cl_E). The following observations are noted: Brain Cl content varies directly with Cl_E and brain Na content in the Cl space (Na_e) varies directly with Na_E, indicating little or no restraint on the inward or outward movement of Na or Cl from the Cl space of brain. The intracellular volume of brain fluid (V_i) derived as the difference between total water and Cl space, decreases with hypernatremia and increases with hyponatremia. The changes in V_i in the acute studies are not accompanied by any change in brain K content, or calculated intracellular Na content, and are approximately 0.6 the changes predicted from osmotic behavior of cells, which apply four assumptions: (*a*) Na_E is proportional to osmolality; (*b*) brain osmolality remains equal to plasma osmolality; (*c*) V_i is osmotically active; and (*d*) there is no net gain or loss of solute from V_i. The validity of these assumptions is considered. When changes in osmolality are sustained, V_i is much closer to control values than when in the acute phase. K content increases in hypernatremia and decreases in hyponatremia. The changes in K content can account for some of the adjustment in V_i observed over the extended period of hyper- or hyponatremia. The regression of $(Na + K)/v$ upon Na_E describes a slope less than 1.0 and an intercept of $(Na + K)/v$ equal to 40% of the control $(Na + K)/v$. These characteristics are interpreted to mean that significant quantities of Na and K in brain are osmotically inactive. The brain protects itself from acute volume changes in response to change in Na_E by the freedom for Na and Cl to move from the Cl space, by V_i not changing acutely to the degree predicted from osmotic properties of cells in general, and by significant quantities of Na + K in V_i being osmotically inactive. With sustained changes in osmolality, V_i approaches normal values and brain K changes to account for part of this later adjustment.

INTRODUCTION

Brain swelling and symptoms of brain dysfunction occur with acutely induced hyponatremia secondary to water retention, i.e. water intoxication, in clinical and experimental settings (1, 2). A decrease in brain volume has been demonstrated with acutely induced hypernatremia (3) secondary to hypertonic saline loading in experimental animals. A similar phenomenon appears to occur in infants with hypernatremia (4). A transient decrease in brain volume is a well-established phenomenon, consequent to the infusion of hypertonic solutions (5).

Dr. M. Kalayci's present address is the University of Pittsburgh School of Medicine, Pittsburgh, Pa. 15200.

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Experimental studies (3, 6–11) have documented the changes in brain fluid and electrolyte that occur with changes in osmolality that were specific for each study. The results have usually shown a change in brain Cl and Na content in the direction of the change in concentration of Cl and Na induced in plasma. Changes in K content in the brain have been noted in some experiments (6–9) but not in others (3, 10, 11). Except for the early study by Yannet (6), there has been little effort to generalize a quantitative relation between changes in plasma osmolality to changes in brain tissue water and electrolytes.

The effects of changes in plasma osmolality upon brain fluid and electrolyte depend upon the rate with which brain water equilibrates with plasma water when a gradient in water potential develops, and the degree to which brain solute content is altered, since the latter influences the final distribution of water. Because rates of exchange for water and solute vary, time is an important element in characterizing changes in brain-fluid volume. This is evident in clinical experience: hypertonic urea injections decrease brain volume, because urea diffusion into brain water is relatively slow and osmotic redistribution of water continues until urea equilibrium is reestablished; rapid reduction of plasma urea concentration in uremia by hemodialysis leads to transitory signs of brain swelling (12) for the same reason. It is also evident that those patients who develop hyponatremia slowly have fewer symptoms of central nervous system dysfunction than do those who develop it rapidly (13, 14).

The studies reported here are designed to compare the effects of acute changes in plasma osmolality with those of sustained changes upon the Na and Cl content of the brain, the fluid phases of the brain, the K content, and the ratio of cation to water in brain fluid. In particular, we have compared the effects of changing osmolality upon observed cell volume with that predicted from the assumption that cell volume is a reciprocal function of osmolality (15).

METHODS

Two studies were carried out in young male Sprague Dawley rats weighing 200–350 g. The experimental protocols for each experimental group in the two studies are as follows.

Study I (Groups 1–5)

Group 1. 13 normal rats fed an ordinary laboratory diet were sacrificed as normal controls.

Group 2. Nine rats were made acutely hypernatremic by intravenous infusion with a solution of 7.5% NaCl, 0.3 ml/min for 50–60 min, and sacrificed 60 min post-infusion.

Group 3. 11 rats were made hypernatremic over 7 days by substituting 5% saline for their usual drinking water. Since their intake was erratic they were then gavaged to receive 2 ml/day per 100 g of body weight of a 5% saline solution, and were sacrificed after 6–7 days of this regimen.

Group 4. 11 rats were made acutely hyponatremic with a dose of 50 mU of Pitressin and infusion of a solution of 1.5% glucose and 1.5% fructose, 0.3 ml/min per 100 g for 50–75 min until they had gained about 15% of body weight; then they were sacrificed within a few minutes.

Group 5. 12 rats were made hyponatremic over a 3 day period by giving them 50 mU of Pitressin in oil daily, and 5–7 ml of water/100 g of body weight by gavage every 6 hr for the three days; they were then sacrificed.

Study II (Groups A–D)

This study was designed to compare quantitatively similar degrees of hyponatremia of 1½ hr and 24 hr duration. To control the degree of hyponatremia the ureters in all rats were ligated at the beginning of the experiment. No fluid or food was given except that noted in the protocol for each group. Paired controls were studied in each experiment because uremia was a variable, particularly in the 24-hr group.

Group A. 1½-hr control group. Seven rats were infused with isotonic saline, 10 ml/100 g of body weight over a period of 1 hr; 1½ hr later they were sacrificed.

Group B. 1½-hr hyponatremic group. Seven rats were infused with 2% glucose, 15 ml/100 g of body weight over a period of 1 hr; 1½ hr later they were sacrificed.

Group C. 24-hr control group. Seven rats were infused as in Group A, but sacrificed 24 hr after infusion.

Group D. 24-hr hyponatremic group. Eight rats were infused as in Group B, but sacrificed 24 hr after infusion.

The animals were sacrificed by exsanguination from the aorta; the blood obtained was used for serum and red cell analyses. The brain was removed whole, weighed fresh, and weighed again after drying at 105°C in order to obtain water content by the difference. The dry residue was eluted with 10 ml of 0.75 N HNO_3 (16). After 48 hr, aliquots of the eluate were taken for Na and K analysis by flame photometry and Cl analysis with a Cotlove chloridometer (17). In study II, serum glucose was measured by the Somogyi-Nelson method (18), serum urea by the method of Seligson (19), and serum osmolality with a Fiske osmometer. Since the dry solids of brain probably did not vary in these studies, fluid and electrolyte values which are referred to dry weight of brain reflect absolute changes in content.

Symbols and Calculations

The following symbols and calculations modified from earlier studies (6, 16, 20–22) are used in developing the results. Na_E, concentration of Na in extracellular fluid (ECF), derived from serum Na concentration, Na_s, assuming a serum water content of 94% and a Donnan factor of 0.96 $\therefore Na_E = Na_s \times 0.96/0.94$; Cl_E, concentration of Cl in ECF with the same assumptions as obtained for calculating $Na_E \therefore Cl_E = Cl_s \times 1/0.94 \times 1/0.96$; Π_s, the effective osmolality of plasma and ECF, i.e., osmolality corrected for NPN and glucose (22); Na_t and Cl_t, Na and Cl content, respectively, of brain as mEq/100 g of dried weight of brain (100 DWB); K_t or K_i K content of brain as mEq/100 DWB; no correction is made for extracellular K; V_t, volume of total brain water as ml/100 DWB; V_{Cl}, volume of distribution of Cl/100 DWB calculated from the formula $V_{Cl} = Cl_t/Cl_E$; V_i, fluid not in the volume of distribution of Cl and termed the intracellular volume/100 DWB–the difference between V_t and V_{Cl}; Na_e, Na content/100 DWB calculated to be in V_{Cl}, assuming its concentration in V_{Cl} is equal to Na_E; Na_i, intracellular Na content/100 DWB–the difference between Na_t and Na_e; $(Na_t + K_t)/V_t$, ratio of Na plus K content to water content in brain (The term avoids the necessity for making any assumptions about extracellular volume in comparing the cation concentration of brain fluid with Na_E.); $(Na_i + K_i)/V_i$, ratio of the sum of Na and K content in intracellular phase to the water content of the intracellular phase.

The effect of a change in osmolality upon intracellular volume can be predicted (15) by the following equation, given the assumptions cited below:

$$pV_i^x = pV_i^c \times Na_E^c/Na_E^x \qquad (1)$$

where pV_i^x is the intracellular volume after a change in osmolality; pV_i^c is the control intracellular volume; Na_E^c is the control Na_E; and Na_E^x is the new Na_E representing a change in osmolality.

The validity of the prediction depends on the following assumptions: (*1*) Na_E is proportional to the effective extracellular osmolality Π_s (22); (*2*) $\Pi_s = \Pi_B$, where Π_B is intracellular osmolality (15, 23); (*3*) V_i^c is osmotically active water bounded by a semipermeable membrane; (*4*) Intracellular solute content of brain remains unchanged (15).

It has been proposed that intracellular solute is, under some conditions, proportional to intracellular K content, K_i (24). In the experiments where K_i changes, equation 1 to predict pV_i^x is amended as follows:

$$pV_i^x = (pV_i \times Na_E/Na_E^x) \times K_i^x/K_i^c \qquad (2)$$

where K_i^x is the K_i of the experimental group and K_i^c is the K_i of the control group, and the fourth assumption states that intracellular solute content is proportional to K_i.

Regression equations are calculated by the method of least squares. The standard error of the mean, standard error of the slope, and correlation coefficient are calculated by the usual formula adapted for use in the Olivetti Programma 101. Differences between group averages are compared by the standard *t* test.

RESULTS

The means and standard deviations of results for each study are tabulated in Table I and I A for both studies. Regression equations are collected into Table II for reference. Because osmolality was not measured in the first study, and because changes in osmolality were effected only by water loading and NaCl loading, Na_E is used throughout instead of Π_s to reflect changes in Π_s. (Π_s, the effective osmolality, is not affected by uremia (22).)

The regression of Cl_E upon Na_E (Table II) indicates that the change in degree and direction of Cl_E is similar to that of Na_E. The slope of the regression, 1.16, indicates Cl_E is changing slightly more than Na_E, probably due in part to the use of hypertonic saline in the hypernatremic studies, with an attendant dilutional acidosis. The slope of almost 1.0, the correlation coefficient of nearly 0.98, and the standard error of the estimate of < 10 affirm the parallelism of these variables.

The effect of changing Cl_E upon Cl_t and Na_E upon Na_t. The slope of regression for brain Cl content, Cl_t, upon Cl_E is cited in Table II and illustrated in Fig. 1. The principal line is derived only from the data from groups 1–5 (study I) and data are plotted for each individual rat. The slope, 0.121, is very nearly the same as the ratio of Cl_t: Cl_E for the controls (group 1), 0.129, and the intercept is not significantly different from zero. For comparison, the line of regression of Cl_t upon Cl_E for groups A–D (study II) is also presented, without a plot of individual points. The similarities between the regressions of these separate and somewhat different experiments are evident. The slope of the second regression, 0.117, is very nearly the same as that for the first, and the intercept is not significantly different from zero. The correlation coefficient for each is high. It is concluded that Cl_t, brain Cl content in these experiments, varies as Cl_E, and that Cl in brain does not act as an effective solute to increase or decrease brain volume. The change in Cl_E in these experiments is proportional to changes in osmolality. Consequently, it can be stated that the volume of distribution of Cl in brain, V_{Cl}, does not change and can be considered in this sense as extracellular fluid.

TABLE I

Mean Values and SD *for Each Group*

	Na_E	Cl_E	V_t	Na_t	Cl_t	K_i	V_{Cl}	V_i	Na_i	Na_i+K_i	$\frac{Na_t+K_t}{V_t}$	$\frac{Na_i+K_i}{V_i}$
	mEq/liter			*ml or mEq/100 g DS*							*mEq/liter*	
Study I												
1. Control	141.5	112.5	361	22.49	14.57	43.29	129	231	4.18	47.46	183	205
n=14	±3.2	±3.4	±7	±0.90	±0.65	±1.03	±3	±5	±0.53	±1.25	±3	±6
Hypernatremic												
2. 3 hr	200.0	198.7	311*	30.24	23.35	44.48§	117*	194*	6.72	51.20	240	265
n=9	±9.4	±14.0	±7	±2.90	±3.05	±0.77	±9	±12	±0.95	±1.37	±13	±19
3. 7 days	180.8	157.9	371‡	30.43	22.47	44.97§	142*	229‡	4.71	49.66	204	218
n=11	±12.1	±11.3	±19	±1.64	1.61	±1.40	±7	±16	±1.00	±1.26	±11	±14
Hyponatremic												
4. 3 hr	115.2	94.9	388*	18.73	11.59	43.48‡	122*	266*	4.66	48.16	160	181
n=11	±5.5	±4.3	±8	±0.93	±0.51	±1.31	±6	±10	±0.64	±1.70	±5	±8
5. 3 days	95.3	65.0	394*	18.05	8.97	36.37*	139‡	255*	4.95	41.32	138	163
n=12	±10.4	±9.9	±8	±1.60	±1.33	±1.54	±18	±21	±1.03	+2.26	±7	±16
Study II												
A Control, 1½ hr	135.3	113.9	368	20.59	13.94	46.51	123	245	4.01	50.54	182	213
n=7	±2.9	±3.1	±10	±0.59	±0.36	±1.75	±5	±13	±0.20	±1.88		±9
B Hyponatremic, 1½ hr	109.1	96.4	397*	17.81	10.63	45.54‡	110§	286*	5.76	51.30	160	179
n=7	±4.1	±4.0	±10	±0.68	±0.38	±1.64	±8	±16	±1.07	±2.45		±6
C Control, 24 hr	138.3	121.3	353	20.34	14.06	46.33	116	237	4.31	50.26	189	213
n=7	±4.6	±6.5	±8	±0.82	+0.74	±1.62	±2	±9	±1.01	±2.10		±9
D Hyponatremic, 24 hr	106.3	93.1	377*	17.79	10.84	44.20§	117‡	260§	5.39	49.58	164	191
n=8	±4.1	±7.9	±9	±0.60	±0.61	±1.03	±8	±14	±0.96	±1.30		±10

P values compared with appropriate control.
* < 0.1%.
‡ not significant.
‖ < 5; >1%.
§ < 1; >0.1%

TABLE I A

Plasma Osmolality, Urea, Glucose Concentration, and Corrected Osmolality in Study II*

	II	Urea	Glucose	II'*
	mOsm/kg	*mmoles/liter*		*mOsm/kg*
Control, 1½ hr	304	5.5	12	287
	±9	±2.1	±2	
Hyponatremia, 1½ hr	264	5.4	15	244
	±12	±2.4	±3	
Control, 24 hr	343	25.0	8	311
	±14	±7	±1	
Hyponatremia, 24 hr	279	17.4	10	252
	±7	±2.4	±3	

* II', or corrected osmolality, equals II, urea (mmoles/liter), and glucose (mmoles/liter).

In control group 1, study I (Table I), with the assumptions described in the calculations, most of brain Na content, Na_t, is in V_{Cl}. Na_t for this group is 22.49 mEq/100 DWB; of this, 18.31 mEq is in V_{Cl} (Na_e) and only 4.18 mEq is in intracellular fluid, Na_i. When the regression of total brain Na, Na_t, upon Na_E is analyzed, the slope (Table II)is very nearly the same, 0.133, as that noted for Cl_t upon Cl_E, 0.121. The intercept of this regression is 4.55 mEq, significantly different from zero and very similar to the calculated Na_i for the control group, 4.18. From these relationships, it is concluded that the Na calculated in V_{Cl}, (Na_e) varies just as Na_E, and Na_e does not act to increase or decrease brain volume under the conditions of these studies.

The Na_i calculated for all the groups varies but little, ranging from 4.01 to 6.72 mEq/100 DWB. Although the total amount is small, Na_i may act as intracellular solute to change brain volume as osmolality changes. This will be true only if Na_i is osmotically active.

All of these effects appear to be true for the acute as well as for the chronic experiments, so that no distinction is made between them. A possible exception is group 2 (acute hypernatremia) where the gain in Cl_t and Na_t in relation to the control is slightly less than the increment in Cl_E and Na_E.

The results of this analysis lead to the prediction that V_{Cl} calculated for each experimental group will not vary inversely to Cl_E or Na_E, as it would if Na and Cl were restrained within the

TABLE II

*Correlation Coefficient and Regression Equations**

Groups	X	Y	$\bar{X}$	$\bar{Y}$	Equation	SE EST	SE SLOPE	r	n
1–5	Na_E	Cl_E	143.4	121.3	$Cl_E = 1.16 \cdot Na_E - 45.6$	9.4	0.07	0.979	58
1–5	Cl_E	Cl_t	121.3	15.71	$Cl_t = 0.121\ Cl_E + 1.02$	1.69	0.0045	0.958	58
A–D	Cl_E	Cl_t‡	105.7	12.31	$Cl_t = 0.117\ Cl_E - 0.05$	0.80	0.010	0.893	29
1–5	Na_E	Na_t	143.4	23.58	$Na_t = 0.133\ Na_E + 4.55$	1.97	0.007	0.935	58
1, 2, 4 A, B	$\frac{141.5 \times 100}{Na_E}$	V_i§	1.05	242.2	$V_i = 145 \cdot 141.5/Na_E + 89.8$	13.1	9.3	0.916	48
1–5	Na_E	$(Na_t+K_t)/V_t$	143.4	182.2	$(Na_t+K_t)/V_t = 0.86 \cdot Na_E + 58.9$	9.8	0.033	0.960	58
A–D	Na_E	$(Na_t+K_t)/V_t$‡	121.7	173.6	$(Na_t+K_t)/V_t = 0.73 \cdot Na_E + 84.2$	6.7	0.082	0.861	29
1–5	Na_E	$(Na_i+K_i)/V_i$	143.4	203.8	$(Na_i+K_i)/V_i = 0.81 \cdot Na_E + 87.1$	16.6	0.506	0.886	58

* All regression analysis done on study I only, unless otherwise stated.

‡ Study II, data only for comparison with study I.

§ Acute experiments and controls of studies I and II; no chronic experiments.

brain and acted as an effective solute to change brain volume. The actual values for the averages of V_{Cl} for each experimental group are noted in Table I. Although average V_{Cl} for some experimental groups is significantly different from their controls, the differences are small. In only one instance (group 2, study I) is the change in V_{Cl} statistically significant and reciprocal to the change in Na_E.

The effect of acute change in Na_E upon V_t. Since brain solids are not affected in these experiments and V_{Cl} changes very little, the changes in brain volume are due almost entirely to changes in the calculated intracellular fluid, V_t, and these changes in V_t are reciprocal to the changes in Na_E.

A regression of V_t upon the reciprocal of Na_E[1] has been derived. Since K is the principal solute in V_t, and since it does not change in the acute experiments (groups 2, 4, and B), but does in the chronic or sustained experiments (groups 3, 5, and D), the regression analysis of V_t upon $141.5/Na_E$ is derived from the data in the acute experiments of both studies (groups 1, 2, 4, A, and B) where K content does not change (equation 1 and assumptions). The results are cited in Table II and Fig. 2, in which the calculated line of regression and lines defining 95% confidence limits of the data are illustrated. The observed line of regression lies between a line representing V_t predicted from equation 1, and one which defines V_t at its control value. The slope of the line derived from equation 1 is 231, which is the control V_t, since control osmolality has been corrected to 1.0 and the intercept by definition is zero. The slope of the regression is 145, and the intercept is 89.8 or 90 ml, approximately 40% of the control V_t. The difference between the theoretical slope, 231, and the observed slope, 145, is significant (0.001). The observed changes in V_t defined by the regression equation depart significantly from the V_t predicted by equation 1 and the assumptions inherent in it. However, the pattern of change in V_t as reciprocal to Na_E is evident and apparently linear. The correlation coefficient is high, 0.92. The ratio of the observed slope to that calculated from equation 1 is 0.62 and is an approximation of the degree to which the assumptions in equation 1 have applied.

The changes in V_t in the chronic experiments. In groups 3, 5 (study I), and D (study II), the changes in Na_E were sustained 24 hr or more. In each, K_t changed significantly from its control. The individual values for V_t plotted against $141.5/Na_E$ for these chronic groups are compared (Fig.

[1] In order to visualize the magnitude and direction of changes in osmolality, the reciprocal of Na_E for each animal is multiplied by the average control Na_E, 141.5. In this way, all deviations in osmolality are related to a control osmolality of 1.0. The reciprocal of osmolality expressed in this way should be a linear function of V_t.

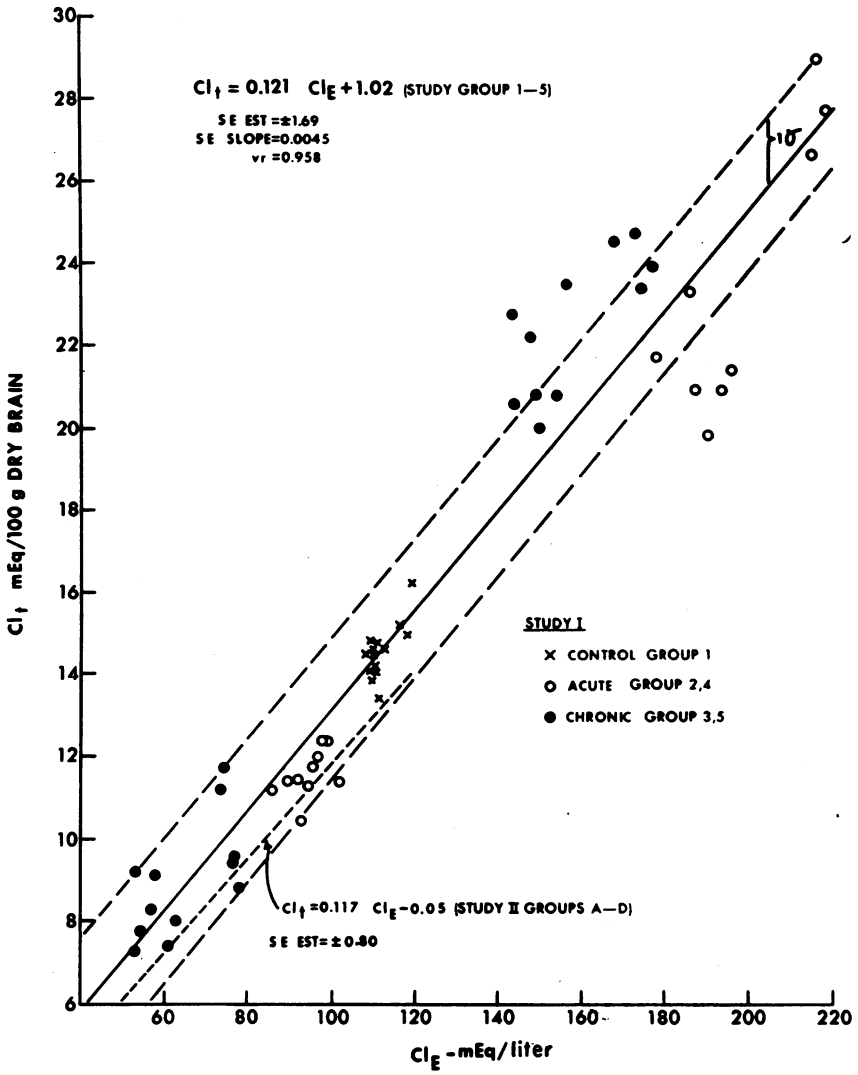

FIGURE 1 Data from study I illustrate the regression of Cl_t upon Cl_E.

3) with the regression lines derived from the acute experiments (see Fig. 2) and their 95% confidence limits. Much of the data from the chronic experiments (groups 3, 5, and D) falls outside the 95% confidence limits. In several instances, despite large changes in osmolality and Na_E, the observed V_t is the same as the control V_t (horizontal line); in these cases, brain volume attains its control size despite a sustained change in osmolality.

The capacity of the brain to restore its volume toward control values after the acute response may be, in part, related to its capacity to alter solute content. The principal solute, K, changes in each of the chronic experiments. In chronic hypernatremia, when V_t is increasing toward normal, K_t increases by 4% (group 3). In chronic hyponatremia, when V_t is decreasing toward normal, K_t decreases 16% (group 5), and 4% (group D). The V_t predicted from equation 2 [2] for group 5 is 288 ml[2] compared with the observed V_t of 255 ml, and a V_t predicted from equation 1 [3] of 345. The correction derived from the decrease in K_t brings the pV_t^x closer to that observed, but not equal to it. If, however, the pV_t^x for this group is calculated from the actual regression of V_t upon

[2] Equation 2, $pV_t = 231 \times 141.5/95.3 \times 36.37/43.29 = 288$.

[3] Equation 1, $pV_t = 231 \times 141.5/95.3 = 345$.

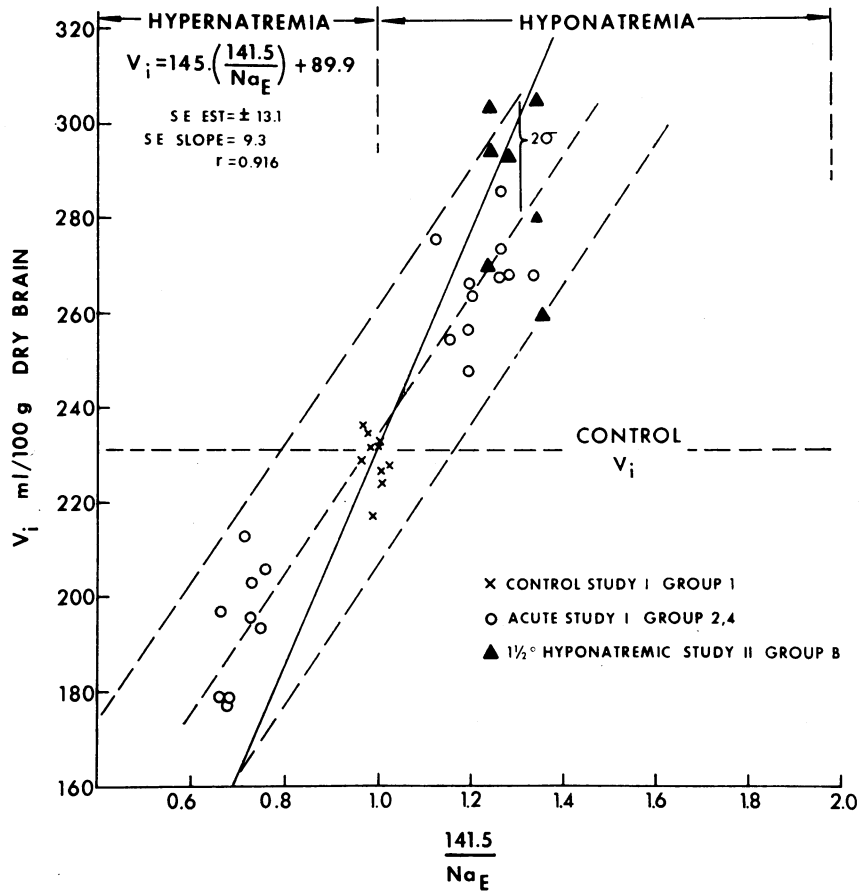

FIGURE 2 Regression of V_i upon the reciprocal of Na_E with data from all the acute studies. The reciprocal of Na_E in each instance is multiplied by the average Na_E of control group 1, so that all changes in the reciprocal of Na_E ($141.5/Na_E$) are related to a control value of 1.0. Hypernatremia is < 1.0, hyponatremia is > 1.0. The solid line describes plot derived from equation 1 of V_i upon $141.5/Na_E$, so that the plot is linear, the slope is equal to the control V_i, and the intercept is zero. The horizontal line describes V_i as constant with changing $141.5/Na_E$. The actual line of regression (and its 95% confidence limits) lies between the two theoretical lines, closer to that derived from equation 1.

141.5/Na derived from the acute experiments and amended on the assumption that its solute content and, hence, its volume decreases in proportion to the decrease in K_i, the calculated V_i is 256.[4]

This obviously compares well with the observed figure of 255 ml. The errors implicit in these calculations are too great to draw conclusions with any confidence about a quantitative relation between V_i and K_i. However, it is fair to assert that V_i of brain with sustained changes in osmolality has a mechanism for adjusting its volume toward normal from either direction, and that this is associated with change in K content in the appropriate direction.

K_i is not changed in group 4 (acute hyponatremia) but is changed in group 5 (chronic hyponatremia). The change in Na_E in group 5, however, is not only sustained, but is much greater, 95 mEq/liter, than that noted in group 4, 115 mEq/liter. To determine whether the lower K_i in group 5 is due to the greater severity of hyponatremia or to the extended time, a comparison is made in study II, groups B and D when the degree of hyponatremia is the same but the time of hyponatremia is different. With comparable de-

[4] $pV_i = [145 \cdot (141.5/95.3) + 90] \times 36.37/43.29 = 256$ ml.

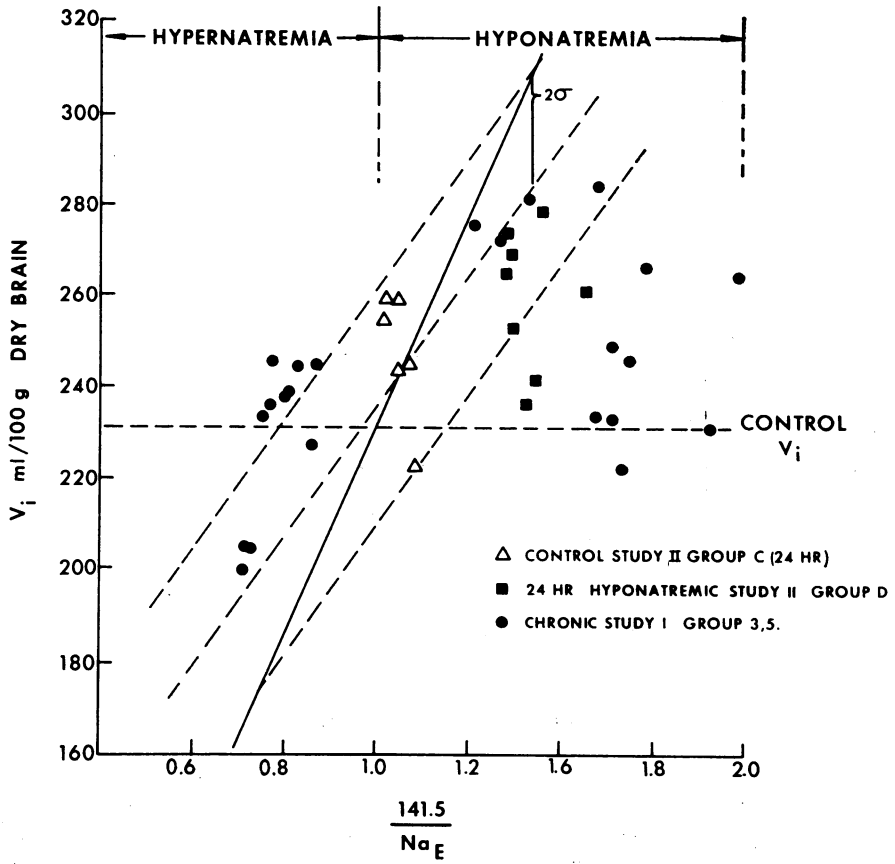

FIGURE 3 The theoretical lines and actual line of regression of V_t upon $141.5/Na_E$ are reproduced, as derived in Fig. 2. The individual points illustrate the relation of V_t to $141.5/Na_E$ for all the chronic experiments of studies I and II, which are compared with the acute. The points are much closer to the control V_t and, in many instances, equal to it despite changes in $141.5/Na_E$. Much of the data falls outside the 95% confidence limits of V_t, derived from the acute studies in Fig. 2.

grees of hyponatremia, K_t shows a definite decrease after 24 hr, but no decrease after 1½ hr, and V_t shows less increase after 24 hr than after 1½ hr. Consequently, time seems to be the important variable in the change in K_t and this change is associated with a lesser change in V_t.

The regression of the ratio of cation content: water content upon Na_E in the brain when Na_E is changing. This relationship is explored to determine the degree to which cation concentration in brain fluid parallels osmolality or Na_E. Since Na_E is manipulated in these experiments, it is the independent variable and the ratio of (Na + K) : V is the dependent variable. Results for the regressions of $(Na_t + K_t) : V_t$ upon Na_E for studies I and II and the details of the regression analysis for each are given in Table II, and the data from study I is illustrated in Fig. 4. The slopes and the intercepts are similar despite the fact that study II contains no rats with hypernatremia and half the rats are uremic. The slope in each instance is less than 1.0. This is true although the ratio of $(Na_t + K_t)/V_t$ is consistently higher than Na_E or $(Na_E + K_E)$ (making any reasonable allowance for K_E). A large positive intercept is evident for both regressions, 58.9 and 84.2, respectively. This intercept is evidence that a significant portion of brain cation is osmotically inactive. The correlation coefficient for each regression is high, 0.96 and 0.86, respectively. This analysis avoids any arbitrary division of cation and fluid into extracellular and intracellular phase.

Since the Na in V_{Cl} (Na_e) has been shown to vary as Na_E (see Methods) and, therefore, to be

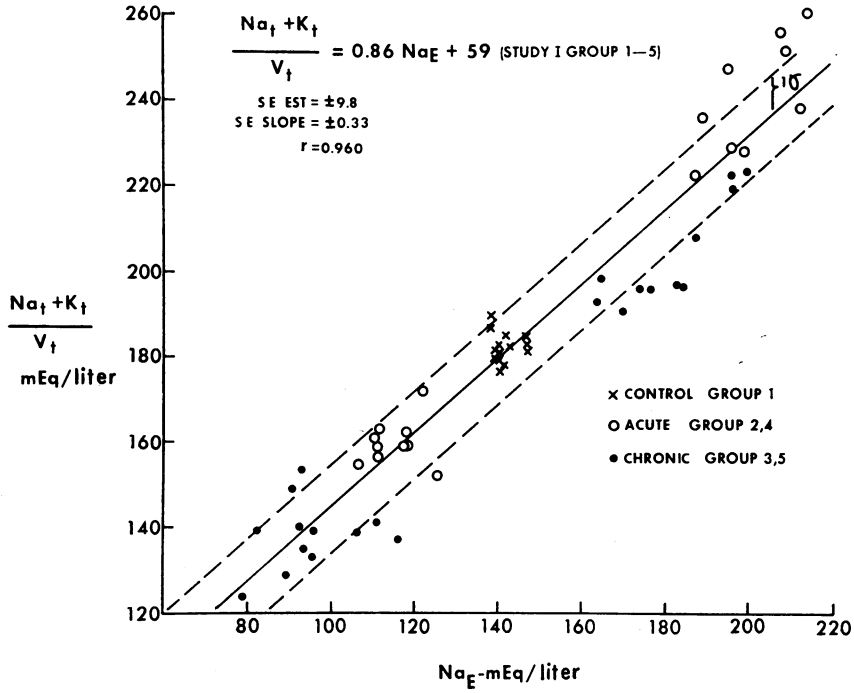

FIGURE 4 Data that illustrate the regression of $Na_t + K_t/V_t$ upon Na_E.

osmotically active, the remaining cation ($Na_t + K_t$) should include all the osmotically inactive cation. Therefore, the regression of $(Na_t + K_t)/V_t$ upon Na_E calculated for study I defines a mean cation concentration in intracellular fluid that is higher–203.8; a slope that is less–0.81; and an intercept that is greater–87.1, than that noted for $(Na_t + K_t)/V_t$ (Table II).

By each analysis, the change in cation concentration in brain water varies, as Na_E, and the correlation is very high. In all three of the derived regressions (Table II), the very appreciable intercept implies that a significant percentage of brain cation is osmotically inactive.

DISCUSSION

The effects of changing Cl_E upon Cl_t and Na_E upon Na_t. Na and Cl in brain and cerebrospinal fluid (CSF) differ from Cl and Na elsewhere in the body in their relation to Cl and Na in plasma. Equilibration of ^{36}Cl and ^{22}Na into brain is slow, 24 hr, compared with its equilibration into interstitial and connective tissue (25, 26). The concentration of Cl and Na in CSF differ from Cl_E and Na_E so that some active transport process must be postulated (27, 28). It would not be surprising if a change in plasma concentration of Cl or Na is not accompanied by a proportional change in brain Cl or Na. Yet in our experiments the changes in brain Cl and Na are proportional, over a wide range of concentration values and within a few hours of time. In other studies this also appears to be the case when examined in specific examples (3, 6–11).

The character and magnitude of the extracellular phase of brain has been the subject of much conflicting information. In early studies, it was assumed that "the Cl space," or V_{Cl}, defined the extracellular phase of brain (6). However, in subsequent studies the values for the volume of distribution of sulfate, thiosulfate, thiocyanate, inulin, and sucrose were all very much lower than V_{Cl} when plasma concentration of the tracer was used in calculating the "extracellular phase" (29–33). The values of each varied, but the extracellular phase of brain was considered to be less than 5% of total brain from these data. Several observations from electronmicrographs were in accord with these low values, since no intercellular spaces could be identified (34, 35).

However, Van Harreveld (36) recently reexamined this issue in detail. He found that impe-

dance measurements of the brain were consistent with an interstitial space much more of the order of 20–30% of brain weight than 4–5% (37). He further demonstrated, by histochemistry, that Cl is in interstitial spaces (38) and, by electron microscopy, that there is a substantial interstitial space (39). This extracellular Cl is absorbed and the interstitial space is obliterated promptly with anoxia. In other studies, the volume of distribution in brain of traditional extracellular markers has been calculated, using concentration in cerebrospinal fluid rather than plasma in the calculation. The results define values for ECF equal to or slightly smaller than those predicted from V_{Cl}. Bourke, Greenberg, and Tower (40) found that the thiocyanate space as calculated was equal to the Cl space and was 30–38% of brain weight. Sucrose and inulin spaces were 22–30% of brain weight, the higher values being observed in larger brains. Other investigators (41, 42) demonstrated that sulfate space was approximately 15–30% of brain weight. The true ECF of brain is probably smaller by 5–10% than the V_{Cl} calculated (36).

Despite a blood-brain barrier separating Na and Cl in plasma from that in brain and CSF, a rapid and proportional change in brain Na and Cl occurs in response to a changing concentration in plasma. We propose, as a possible explanation, that the Na and Cl content of brain is dependent upon a process of filtration or transport of Na or Cl (43) across the blood-brain barrier, in which the Na_E and/or Cl_E is substrate which, within the physiological range, is limiting to the rate of filtration or transport. Within the framework of this proposal the velocity of filtration or transport within the ranges of "substrate" concentration will be linearly related to Na_E and Cl_E. Whichever of the two, Na or Cl, is primarily determinant is not apparent from our experiments. This capacity of brain to regulate Na and Cl content quickly, so that a brain ECF does not change, even in the face of major and rapid change in osmolality, has a valuable effect in limiting the change in brain volume, since brain ECF is between 25 and 30% of total brain weight.

The acute response of V_i to changing Na_E. The responses of V_i to acute changes in osmolality are in the expected directions. Since the observed changes in V_i in the acute studies are less than those predicted by equation 1 (see Methods), the assumptions inherent in this equation are in question, or the true V_i is smaller than the calculated V_i. This latter is unlikely, because this would mean that the calculated V_{Cl} is smaller than the true ECF. The errors in calculating V_{Cl} are likely to predict a V_{Cl} larger than the true ECF, not smaller, (see Discussion) and hence V_i is likely to be underestimated by our calculations, not overestimated.

The first assumption, that of relating II_s to Na_E, probably is correct, since all the changes in osmolality are induced either by water or saline loading, except where uremia coexists. Even then, it is likely that Na_E reflects a better measure of effective osmolality in relation to its effect on cell volume than osmolality measured directly. Although glucose and fructose infusions were used, glycosuria did not occur in any experiment, and in study II plasma glucose concentrations (Table II) did not exceed 15 mOsm/liter, compared with a control of 6 mOsm/liter.

The equality of brain osmolality to II_s, the second assumption, may be less certain. Maffly and Leaf (23), in measuring melting curves of tissues from normal rats, found muscle, liver, and kidney cortex to have a melting curve similar to that of plasma. A slight discrepancy existed for brain but they felt a systematic experimental error could account for the discrepancy. Stern and Coxon (44), who measured the freezing point of quick-frozen brains in rats, found a discrepancy in osmolality between plasma and brain for normal rats. The discrepancy was greater in rats with acute hyponatremia, and less with acute hypernatremia. However, Brodsky, Applebaum, Dennis, Rehm, Miley, and Diamond (45), using a technic similar to that of Stern and Coxon for measuring tissue osmolality, found discrepancies (46) with their technic, compared with a later technic where no osmolar gradients between tissue and plasma existed. Wise (47) reported gradients in osmolality between plasma and CSF in patients who received 3.6 g/kg of 20% mannitol. The gradients gradually dissipated over 2–3 hr after the infusion. From these results, it is possible to infer that osmolar gradients or differences in water potentials may exist between brain tissue and plasma, at least for periods of several hours.

There is also a possibility that some water in the brain is osmotically inactive, contrary to the

third assumption. Water existing as an ice-like lattice crystal on lipid or protein matrix has been described as being osmotically inactive (48). Recently, Savitz, Sidel, and Solomon (49) reported studies in red cells which indicate that as much as 20% of red cell water is osmotically inactive. In our studies, 90 of 230 ml, or nearly 40%, of brain water in normal rats would be osmotically inactive. This is an extraordinary percentage of total water to be osmotically inactive and this is an unlikely explanation by itself.

The loss or gain of solute not mirrored in changes in K_i may exist, contrary to assumption 4. Inspection of Fig. 2 suggests that the discrepancy of observed V_i from that predicted is rather evenly distributed. Consequently the change in solute content of V_i necessary to account for this discrepancy would have to be proportional to the change in Na_E. A mechanism may exist in which osmolality change leads to reciprocal changes in osmotic coefficients, or aggregation of smaller molecular species into larger ones. It has been proposed before, that changes in cation dissociation may occur to effect changes in cell osmolality (16); such a mechanism may apply in our studies.

In summary, it must be concluded that the assumptions (15) underlying equation 1 apply to the brain, but only to effect changes equal to approximately 0.6 that one would predict if the assumptions were quantitatively correct. It is beyond the scope of these studies to determine which of the assumptions must be modified.

The response of V_i to sustained changes in Na_E. In sustained hyponatremia, the changes in K content are not only statistically significant but after 3 days are equal to 16% of total K_i. These losses of K_i are associated with a further loss of cell volume, without any important gains in cell Na (Na_i). The gains in K_i with hypernatremia are small and, quantitatively, could influence V_i but little.

Losses of K_i (approximately 10% of the total) have been noted in previous studies of water loading or salt depletion to induce hyponatremia (6–8), where brains were obtained from 3–48 hr afterward. In the more acute studies, where the brain was removed promptly, no change in brain K was noted (10, 11).

Sotos, Dodge, Meara, and Talbot (9) produced acute hyperosmolality by mannitol loading in three rabbits. This was accompanied by an increase in brain K content; a small increase in K_i in their study may have occurred with acute saline loading. In the study of Finberg, Luttrell, and Redd (3) acute hypernatremia, secondary to acute intraperitoneal injection of hypertonic saline, was not accompanied by change in brain K content.

The differences in K content, both in hypo- and hyperosmolality among the various studies, appear to be partly time-dependent, i.e., in hyponatremia, K loss occurs probably within 24 hr but not within 1–2 hr; in hyperosmolality, K gain may develop within hours, but appears to progress somewhat with time. Our own experiments are in accord with time-dependent losses of K in hyponatremia and gains in hypernatremia, in which the changes develop within hours to days.

These findings point to a mechanism whereby cells–brain cells in this case–can change cell volume toward normal by altering solute content in the form of K. The underlying mechanism affecting this adjustment is obscure. Whether the mechanism described here is a property of most cells or an adaptation of a few cells only, such as those of the CNS, is not yet clear. When muscle and red cells were also examined in our experiments, the red cell K changed in a manner similar to that noted for brain, but muscle cells did not gain or lose K within the time span of our experiments.

The relation of cation concentration in total brain water and in V_i to Na_E. The excellent correlation in our studies between cation concentration in brain fluid and Na_E, and the character of the regression, confirms–in a general way for brain–the earlier relation that was developed between Na concentration in serum and exchangeable Na plus K in total body water (22). However, the intercepts of the three regressions of these data are very much greater than zero. Both Na and K exchange between brain and plasma is slow in comparison with most tissues (26, 50). The positive intercept is present in the equation which involves total brain cations and water, and the slope-intercept seems unaffected by the time variables encompassed by these studies. This finding constitutes strong evidence that as much as 30–45% of brain cation in the control rats is not osmotically active. Since the Na in V_{Cl} is capable of rapid equilibration with Na_E, it seems likely that virtually all the osmotically inactive cation is in V_i.

This fraction might contribute to a changing solute content by changing its degree of dissociation without changing total cation content of the brain. A mechanism of this type has been cited as a possibility in the discussion of the response of V_i to acute changes in Na_E (see Discussion). If the cations of V_i were all active, then the slope of the regressions of their concentrations upon Na_E should be greater than 1.0, and be approximately equal to the ratio of cation concentration to Na_E for the controls.

The properties of the brain governing its response to acute changes in Na_E limit changes in total brain volume to the intracellular phase, which is approximately half that of the total brain volume. The changes in the intracellular phase are limited to two-thirds of those which would occur if V_i in brain behaves according to the assumption in equation 1. The sum of these effects is to reduce changes in brain volume in response to changing Na_E well below the changes that would occur, should total cation and solute content in total brain water remain constant behind a blood-brain barrier that allowed rapid equilibration of any differences in osmolality. The degree of change in brain volume in the face of an acute reduction in Na_E can be calculated from these observations. The value of this in limiting change in brain volume is obvious. The effect can be appreciated from the following example: an acute reduction in Na_E from 138 mEq/liter to 125 mEq/liter predicts an increase in volume of 10% $(138/125 - 1) \times 100$ in that phase of the brain that conforms to the assumption of equation 1. Since V_i is only half that of total brain volume (0.5) and change in V_i is only 0.6 of that predicted from equation 1, the increase in total brain volume is $[100 + (0.5)(0.6)(138/125)100] - 100\%$ or 3%. This degree of displacement has been shown capable of producing symptoms (51). If swelling of this type involved a greater per cent of brain mass, the development of symptomatic cerebral edema secondary to acute hyponatremia would be evident more often. The secondary readjustment of brain volume toward normal with more sustained change in osmolality and associated with a change in brain K content, provides another mechanism for protecting the brain from extreme volume changes. It also provides a reasonable explanation for the tolerance to sustained hypo- and hypernatremia that is observed in clinical experience.

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