

# Factors That Limit Brain Volume

## Changes in Response to Acute and Sustained Hyper- and Hyponatremia

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**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 ( $\text{Na}_E$  and  $\text{Cl}_E$ ). The following observations are noted: Brain Cl content varies directly with  $\text{Cl}_E$  and brain Na content in the Cl space ( $\text{Na}_i$ ) varies directly with  $\text{Na}_E$ , 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)  $\text{Na}_E$  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  $(\text{Na} + \text{K})/v$  upon  $\text{Na}_E$  describes a slope less than 1.0 and an intercept of  $(\text{Na} + \text{K})/v$  equal to 40% of the control  $(\text{Na} + \text{K})/v$ . 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  $\text{Na}_E$  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  $\text{Na} + \text{K}$  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).

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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<sub>3</sub> (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  $Na_E$ .  $\therefore Cl_E = Cl_s \times 1/0.94 \times 1/0.96$ ;  $\Pi_s$ , 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;  $V_{ci}$ , volume of distribution of Cl/100 DWB calculated from the formula  $V_{ci} = Cl_t/Cl_E$ ;  $V_i$ , fluid not in the volume of distribution of Cl and termed the intracellular volume/100 DWB—the difference between  $V_t$  and  $V_{ci}$ ;  $Na_E$ , Na content/100 DWB calculated to be in  $V_{ci}$ , assuming its concentration in  $V_{ci}$  is equal to  $Na_E$ ;  $Na_t$ , 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  $Na_E$ );  $(Na_t + K_t)/V_i$ , 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:

$$pV_i^* = pV_i^c \times Na_E^c/Na_E^* \quad (1)$$

where  $pV_i^*$  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  $Na_E^*$  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  $\Pi_s$  (22); (2)  $\Pi_s = \Pi_E$ , where  $\Pi_E$  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  $pV_i^*$  is amended as follows:

$$pV_i^* = (pV_i \times Na_E/Na_E^*) \times K_i^c/K_i^* \quad (2)$$

where  $K_i^*$  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 IA 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  $\Pi_s$  to reflect changes in  $\Pi_s$ . ( $\Pi_s$ , 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,  $V_{ci}$ , does not change and can be considered in this sense as extracellular fluid.

TABLE I  
Mean Values and SD for Each Group

	Na <sub>E</sub>	Cl <sub>E</sub>	V <sub>t</sub>	Na <sub>t</sub>	Cl <sub>t</sub>	K <sub>i</sub>	V <sub>Cl</sub>	V <sub>i</sub>	Na <sub>i</sub>	Na <sub>i</sub> +K <sub>i</sub>	Na <sub>t</sub> +K <sub>t</sub> V <sub>i</sub>	Na <sub>i</sub> +K <sub>i</sub> V <sub>i</sub>
	mEq/liter			ml or mEq/100 g DS						mEq/liter		
Study I												
1. Control n = 14	141.5 ±3.2	112.5 ±3.4	361 ±7	22.49 ±0.90	14.57 ±0.65	43.29 ±1.03	129 ±3	231 ±5	4.18 ±0.53	47.46 ±1.25	183 ±3	205 ±6
Hypernatremic												
2. 3 hr n = 9	200.0 ±9.4	198.7 ±14.0	311* ±7	30.24 ±2.90	23.35 ±3.05	44.48§ ±0.77	117* ±9	194* ±12	6.72 ±0.95	51.20 ±1.37	240 ±13	265 ±19
3. 7 days n = 11	180.8 ±12.1	157.9 ±11.3	371‡ ±19	30.43 ±1.64	22.47 1.61	44.97§ ±1.40	142* ±7	229‡ ±16	4.71 ±1.00	49.66 ±1.26	204 ±11	218 ±14
Hyponatremic												
4. 3 hr n = 11	115.2 ±5.5	94.9 ±4.3	388* ±8	18.73 ±0.93	11.59 ±0.51	43.48‡ ±1.31	122* ±6	266* ±10	4.66 ±0.64	48.16 ±1.70	160 ±5	181 ±8
5. 3 days n = 12	95.3 ±10.4	65.0 ±9.9	394* ±8	18.05 ±1.60	8.97 ±1.33	36.37* ±1.54	139‡ ±18	255* ±21	4.95 ±1.03	41.32 ±2.26	138 ±7	163 ±16
Study II												
A Control, 1½ hr n = 7	135.3 ±2.9	113.9 ±3.1	368 ±10	20.59 ±0.59	13.94 ±0.36	46.51 ±1.75	123 ±5	245 ±13	4.01 ±0.20	50.54 ±1.88	182 ±9	213 ±9
B Hyponatremic, 1½ hr n = 7	109.1 ±4.1	96.4 ±4.0	397* ±10	17.81 ±0.68	10.63 ±0.38	45.54‡ ±1.64	110§ ±8	286* ±16	5.76 ±1.07	51.30 ±2.45	160 ±6	179 ±6
C Control, 24 hr n = 7	138.3 ±4.6	121.3 ±6.5	353 ±8	20.34 ±0.82	14.06 ±0.74	46.33 ±1.62	116 ±2	237 ±9	4.31 ±1.01	50.26 ±2.10	189 ±9	213 ±9
D Hyponatremic, 24 hr n = 8	106.3 ±4.1	93.1 ±7.9	377* ±9	17.79 ±0.60	10.84 ±0.61	44.20§ ±1.03	117‡ ±8	260§ ±14	5.39 ±0.96	49.58 ±1.30	164 ±10	191 ±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 ±9	5.5 ±2.1	12 ±2	287
Hyponatremia, 1½ hr	264 ±12	5.4 ±2.4	15 ±3	244
Control, 24 hr	343 ±14	25.0 ±7	8 ±1	311
Hyponatremia, 24 hr	279 ±7	17.4 ±2.4	10 ±3	252

\* 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<sub>t</sub>, is in V<sub>Cl</sub>. Na<sub>t</sub> for this group is 22.49 mEq/100 DWB; of this, 18.31 mEq is in V<sub>Cl</sub> (Na<sub>e</sub>) and only 4.18 mEq is in intracellular fluid, Na<sub>i</sub>. When the regression of total brain Na, Na<sub>t</sub>, upon Na<sub>E</sub> is analyzed, the slope (Table II) is very nearly the same, 0.133, as that noted for Cl<sub>t</sub> upon Cl<sub>E</sub>, 0.121. The intercept of this

regression is 4.55 mEq, significantly different from zero and very similar to the calculated Na<sub>i</sub> for the control group, 4.18. From these relationships, it is concluded that the Na calculated in V<sub>Cl</sub>, (Na<sub>e</sub>) varies just as Na<sub>E</sub>, and Na<sub>e</sub> does not act to increase or decrease brain volume under the conditions of these studies.

The Na<sub>i</sub> 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<sub>i</sub> may act as intracellular solute to change brain volume as osmolality changes. This will be true only if Na<sub>i</sub> 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<sub>t</sub> and Na<sub>t</sub> in relation to the control is slightly less than the increment in Cl<sub>E</sub> and Na<sub>E</sub>.

The results of this analysis lead to the prediction that V<sub>Cl</sub> calculated for each experimental group will not vary inversely to Cl<sub>E</sub> or Na<sub>E</sub>, 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		r	n
						EST	SLOPE		
1-5	Na <sub>E</sub>	Cl <sub>E</sub>	143.4	121.3	Cl <sub>E</sub> = 1.16 · Na <sub>E</sub> - 45.6	9.4	0.07	0.979	58
1-5	Cl <sub>E</sub>	Cl <sub>i</sub>	121.3	15.71	Cl <sub>i</sub> = 0.121 Cl <sub>E</sub> + 1.02	1.69	0.0045	0.958	58
A-D	Cl <sub>E</sub>	Cl <sub>i</sub> †	105.7	12.31	Cl <sub>i</sub> = 0.117 Cl <sub>E</sub> - 0.05	0.80	0.010	0.893	29
1-5	Na <sub>E</sub>	Na <sub>i</sub>	143.4	23.58	Na <sub>i</sub> = 0.133 Na <sub>E</sub> + 4.55	1.97	0.007	0.935	58
1, 2, 4 A, B	$\frac{141.5 \times 100}{\text{Na}_E}$	V <sub>i</sub> §	1.05	242.2	V <sub>i</sub> = 145 · 141.5/Na <sub>E</sub> + 89.8	13.1	9.3	0.916	48
1-5	Na <sub>E</sub>	(Na <sub>i</sub> + K <sub>i</sub> )/V <sub>i</sub>	143.4	182.2	(Na <sub>i</sub> + K <sub>i</sub> )/V <sub>i</sub> = 0.86 · Na <sub>E</sub> + 58.9	9.8	0.033	0.960	58
A-D	Na <sub>E</sub>	(Na <sub>i</sub> + K <sub>i</sub> )/V <sub>i</sub> †	121.7	173.6	(Na <sub>i</sub> + K <sub>i</sub> )/V <sub>i</sub> = 0.73 · Na <sub>E</sub> + 84.2	6.7	0.082	0.861	29
1-5	Na <sub>E</sub>	(Na <sub>i</sub> + K <sub>i</sub> )/V <sub>i</sub>	143.4	203.8	(Na <sub>i</sub> + K <sub>i</sub> )/V <sub>i</sub> = 0.81 · Na <sub>E</sub> + 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<sub>Cl</sub> for each experimental group are noted in Table I. Although average V<sub>Cl</sub> 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<sub>Cl</sub> statistically significant and reciprocal to the change in Na<sub>E</sub>.

*The effect of acute change in Na<sub>E</sub> upon V<sub>i</sub>.* Since brain solids are not affected in these experiments and V<sub>Cl</sub> changes very little, the changes in brain volume are due almost entirely to changes in the calculated intracellular fluid, V<sub>i</sub>, and these changes in V<sub>i</sub> are reciprocal to the changes in Na<sub>E</sub>.

A regression of V<sub>i</sub> upon the reciprocal of Na<sub>E</sub><sup>1</sup> has been derived. Since K is the principal solute in V<sub>i</sub>, 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<sub>i</sub> upon 141.5/Na<sub>E</sub> 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 as-

sumptions). 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<sub>i</sub> predicted from equation 1, and one which defines V<sub>i</sub> at its control value. The slope of the line derived from equation 1 is 231, which is the control V<sub>i</sub>, 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<sub>i</sub>. The difference between the theoretical slope, 231, and the observed slope, 145, is significant (0.001). The observed changes in V<sub>i</sub> defined by the regression equation depart significantly from the V<sub>i</sub> predicted by equation 1 and the assumptions inherent in it. However, the pattern of change in V<sub>i</sub> as reciprocal to Na<sub>E</sub> 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<sub>i</sub> in the chronic experiments.*

In groups 3, 5 (study I), and D (study II), the changes in Na<sub>E</sub> were sustained 24 hr or more. In each, K<sub>i</sub> changed significantly from its control. The individual values for V<sub>i</sub> plotted against 141.5/Na<sub>E</sub> for these chronic groups are compared (Fig.

<sup>1</sup> In order to visualize the magnitude and direction of changes in osmolality, the reciprocal of Na<sub>E</sub> for each animal is multiplied by the average control Na<sub>E</sub>, 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<sub>i</sub>.

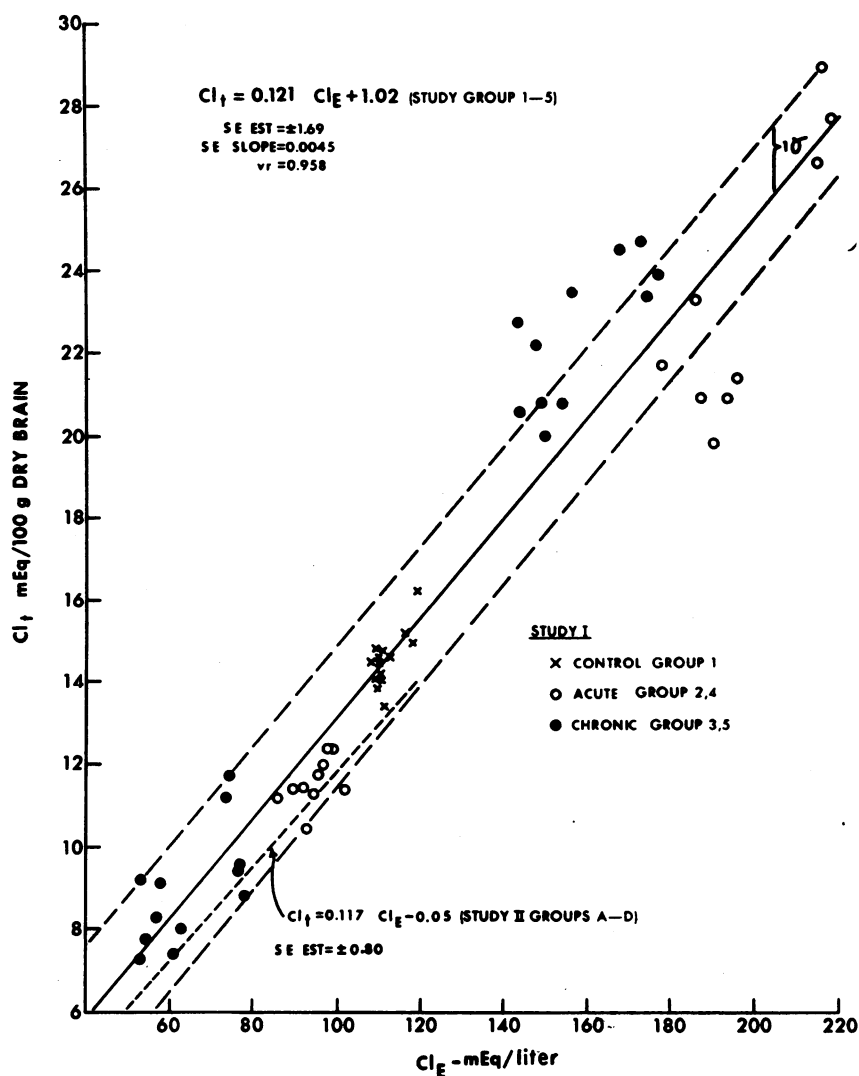


FIGURE 1 Data from study I illustrate the regression of  $Cl_B$  upon  $Cl_E$ .

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  $V_i$  is the same as the control  $V_i$  (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 hyperna-

tremia, when  $V_i$  is increasing toward normal,  $K_i$  increases by 4% (group 3). In chronic hyponatremia, when  $V_i$  is decreasing toward normal,  $K_i$  decreases 16% (group 5), and 4% (group D). The  $V_i$  predicted from equation 2<sup>2</sup> for group 5 is 288 ml<sup>2</sup> compared with the observed  $V_i$  of 255 ml, and a  $V_i$  predicted from equation 1<sup>3</sup> of 345. The correction derived from the decrease in  $K_i$  brings the  $pV_i$ <sup>2</sup> closer to that observed, but not equal to it. If, however, the  $pV_i$ <sup>2</sup> for this group is calculated from the actual regression of  $V_i$  upon

<sup>2</sup> Equation 2,  $pV_i = 231 \times 141.5/95.3 \times 36.37/43.29 = 288$ .

<sup>3</sup> Equation 1,  $pV_i = 231 \times 141.5/95.3 = 345$ .

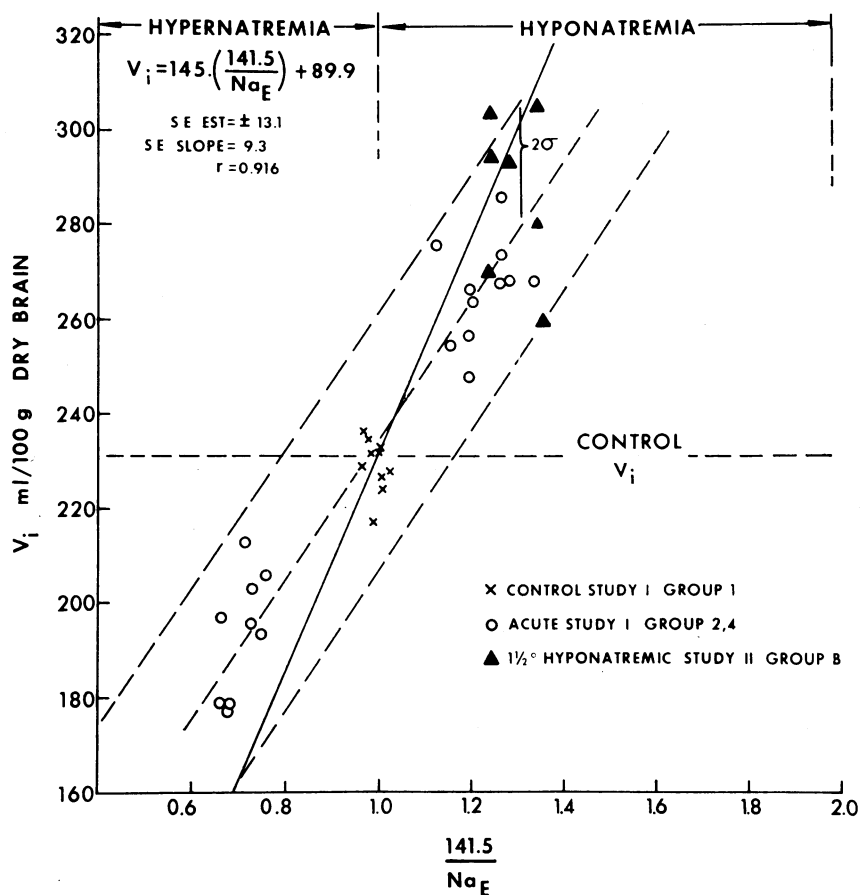


FIGURE 2 Regression of  $V_i$  upon the reciprocal of  $Na_E$  with data from all the acute studies. The reciprocal of  $Na_E$  in each instance is multiplied by the average  $Na_E$  of control group 1, so that all changes in the reciprocal of  $Na_E$  ( $141.5/Na_E$ ) are related to a control value 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.<sup>4</sup>

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 as-

sociated 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-

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

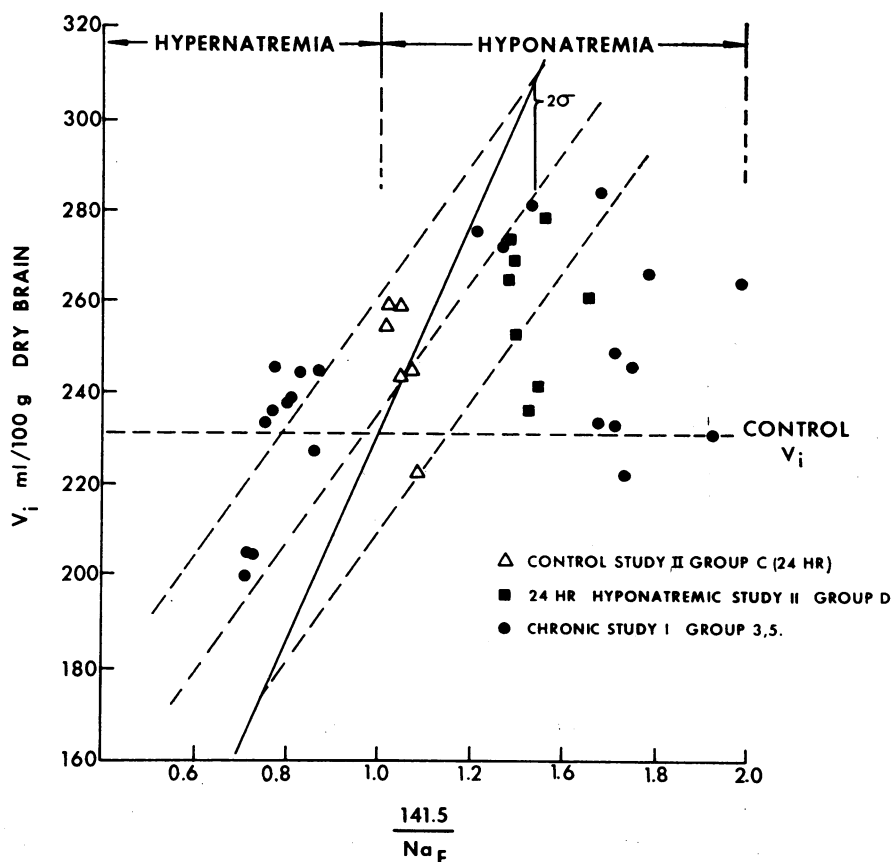


FIGURE 3 The theoretical lines and actual line of regression of  $V_i$  upon  $141.5/Na_E$  are reproduced, as derived in Fig. 2. The individual points illustrate the relation of  $V_i$  to  $141.5/Na_E$  for all the chronic experiments of studies I and II, which are compared with the acute. The points are much closer to the control  $V_i$  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  $V_i$ , derived from the acute studies in Fig. 2.

degrees of hyponatremia,  $K_i$  shows a definite decrease after 24 hr, but no decrease after  $1\frac{1}{2}$  hr, and  $V_i$  shows less increase after 24 hr than after  $1\frac{1}{2}$  hr. Consequently, time seems to be the important variable in the change in  $K_i$  and this change is associated with a lesser change in  $V_i$ .

*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  $(Na_i + K_i) : V_i$  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  $(Na_i + K_i)/V_i$  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

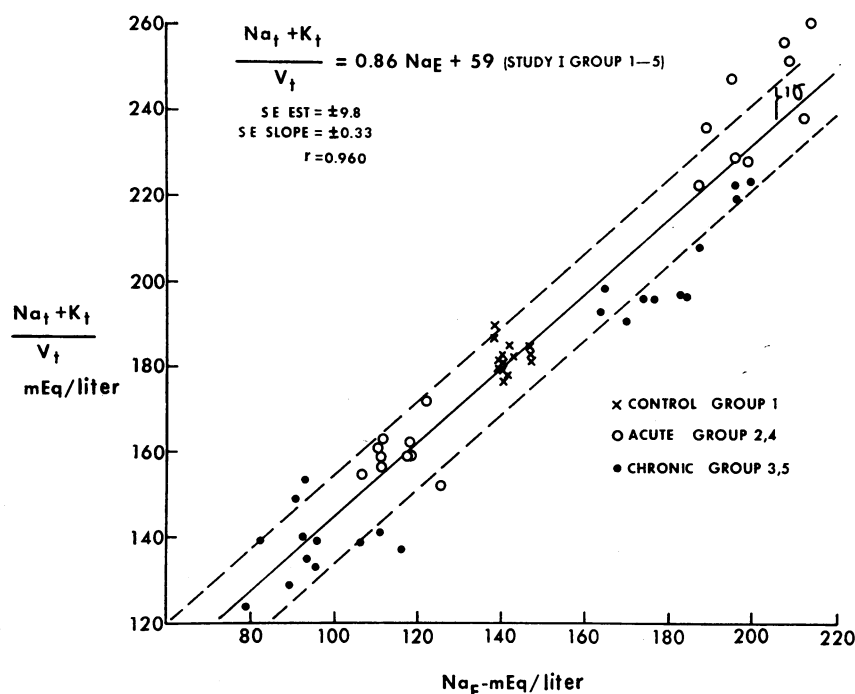


FIGURE 4 Data that illustrate the regression of  $\text{Na}_i + \text{K}_i/V_i$  upon  $\text{Na}_E$ .

osmotically active, the remaining cation ( $\text{Na}_i + \text{K}_i$ ) should include all the osmotically inactive cation. Therefore, the regression of  $(\text{Na}_i + \text{K}_i)/V_i$  upon  $\text{Na}_E$  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  $(\text{Na}_i + \text{K}_i)/V_i$  (Table II).

By each analysis, the change in cation concentration in brain water varies, as  $\text{Na}_E$ , 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  $\text{Cl}_E$  upon  $\text{Cl}_i$  and  $\text{Na}_E$  upon  $\text{Na}_i$ .* 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  $^{36}\text{Cl}$  and  $^{22}\text{Na}$  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  $\text{Cl}_E$  and  $\text{Na}_E$  so that some active transport process must be postulated (27, 28). It would not be sur-

prising 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  $V_{\text{Cl}}$ , 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  $V_{\text{Cl}}$  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 re-examined 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  $V_{OI}$  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  $\text{Na}_E$  (see Discussion). If the cations of  $V_i$  were all active, then the slope of the regressions of their concentrations upon  $\text{Na}_E$  should be greater than 1.0, and be approximately equal to the ratio of cation concentration to  $\text{Na}_E$  for the controls.

The properties of the brain governing its response to acute changes in  $\text{Na}_E$  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  $\text{Na}_E$  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  $\text{Na}_E$  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  $\text{Na}_E$  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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