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Effects of hypernatraemia in the central nervous system and its therapy in rats and rabbits

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1. We studied the effects of acute (1 or 4 h) and chronic (1 week) hypernatraemia (plasma [Na^+], 170–190 mM) on brain histology, and brain water and solute contents in rats and rabbits.

2. In rabbits with acute hypernatraemia, there was significant loss of intracelluar brain water, with increases in brain [$Na^+ + K^+$], amino acid concentration, and undetermined solute (idiogenic osmole). After 1 week of recovery, brain intracellular water content had returned to normal.

3. In hypernatraemic rats there was myelinolysis of brain white matter, with karyorrhexis and necrosis of neurons.

4. Hypernatraemic rabbits were treated with 77 mM NaCl (I.V.) to normalize plasma [Na^+] over 4–24 h intervals. Therapy of either acute or chronic hypernatraemia resulted in significant brain oedema because brain osmolality failed to decrease at the same rate as plasma osmolality.

5. It is concluded that: (a) untreated hypernatraemia results in brain lesions demonstrating myelinolysis and cellular necrosis; (b) normalization of hypernatraemia over 4–24 h results in cerebral oedema, due primarily to failure of brain amino acids and idiogenic osmoles to dissipate as plasma [Na^+] is decreased to normal.

Hypernatraemia is a common clinical condition which tends to affect individuals at the extremes of age – children and the elderly – and frequently leads to central nervous system damage or death. Plasma Na^+ levels associated with brain damage in hypernatraemic patients have been in the range 160–190 mM (Zierler, 1958; Finberg, Kiley & Luttrell, 1963; Morris-Jones, Houston & Evans, 1967; Snyder, Feigal & Arieff, 1987). Previous studies on animals from both our laboratory and others suggest that central nervous system damage or death can occur as a consequence of untreated hypernatraemia (Finberg *et al.* 1963; Morris-Jones *et al.* 1967; De Villota, Cavanilles & Stein, 1973; Simmons, Adcock, Bard & Battaglia, 1974; Snyder *et al.* 1987), but the mechanisms responsible are unclear.

Improper therapy of hypernatraemia can also produce brain dysfunction, neurological deterioration and death (Lutrell & Finberg, 1959; Hogan, Dodge & Gill, 1969). More recent information suggests that the adaptive process of the brain to chronic hypernatraemia may serve to protect the brain from further damage by *de novo* production of solutes that might tend to restore brain cell volume towards normal (Heilig, Stromski, Blumenfeld, Lee & Gullans, 1989; Lien, Shapiro & Chan, 1990). However, since these studies did not evaluate brain osmolality, the contribution of these *de novo* solutes to brain volume regulation remains unknown. Moreover, the accumulation of these potentially osmotically active solutes, while protecting brain volume during the induction of the hyperosmolar state, might produce detrimental effects as a result of cerebral oedema occurring during therapy of hypernatraemia (Lutrell & Finberg, 1959; Hogan, Pickering, Dodge, Shepard & Master, 1984). The effects of therapy of hypernatraemia on dissipation of undetermined solutes (idiogenic osmoles) and resultant brain osmolality have not been studied. The optimal therapy for hypernatraemia is thus not known, and currently, mortality remains high in both children (20–30%) and adults (40–50%, Snyder *et al.* 1987). In the UK, there are multiple reports of death or permanent brain damage as a consequence of lack of knowledge of definitive therapy for the disorder in children (e.g. Morris-Jones *et al.* 1967; Macaulay & Watson, 1967).

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Some anatomical abnormalities, cerebral haemorrhage or thrombosis, have been reported in the brains of patients and animals who have died with hypernatraemia (Finberg, Luttrell & Redd, 1959; Morris-Jones *et al.* 1967). There is information in the literature suggesting that hypernatraemia can induce demyelinative lesions in humans (Ayus, Krothapalli & Arieff, 1987; McKee, Winkelman & Banker, 1988; Clark, 1992). However, the hypernatraemia in these patients was often associated with other medical illness (burns, alcoholism) commonly associated with cerebral demyelination. No information exists as to the role of hypernatraemia *per se* in the production of these brain lesions.

The present study was therefore undertaken to evaluate the effects of experimental hypernatraemia, and its treatment, on brain adaptation and histology in two different animal species.

METHODS

Studies in rabbits

The experimental protocol was approved by the Animal Studies Subcommittee (Veterans Administration Medical Center, San Francisco, CA, USA). Rabbits were used to study the effects of hypernatraemia on brain water and solute content because in this species grey matter can be readily isolated, and also because of our substantial experience in brain analysis using rabbit models of fluid and electrolyte disorders (Arieff, Doerner, Zelig & Massry, 1974*a*; Arieff, Kleeman, Keushkerian & Bagdoyan, 1974*b*). Studies were carried out on New Zealand White rabbits (3–4 kg) of both genders, in which hypernatraemia was induced either acutely for periods of 1 or 4 h, or chronically for 7 days. Hypernatraemic rabbits were then treated over intervals of 4–24 h, to return the plasma $[Na^+]$ to normal values (below 150 mM).

The following goups of rabbits were studied.

Group I: effects of acute untreated hypernatraemia on brain adaptation

In preliminary studies on conscious rabbits, not reported here, it was noted that infusion of hypertonic NaCl (1·7 M) sometimes resulted in slowing of respiration. Therefore, all of these experiments were performed on rabbits that were anaesthetized, paralysed and artificially ventilated (Arieff, Kleeman, Keushkerian & Bagdoyan, 1973). Briefly, twelve control and nine experimental rabbits, which were to be made acutely hypernatraemic (see below), were fasted overnight. Each animal was placed in a restraining box, an ear vein was catheterized via hypodermic needle insertion under 2% lidocaine (lignocaine) analgesia, and anaesthesia (sodium pentobarbitone, 20 mg kg^{-1} I.V.) was induced via this catheter which was left *in situ*. After removing the rabbit from the box, it was placed on a heating blanket (37 °C) and tracheostomized. A 20 gauge catheter (Angiocath; Quinton Instrument Company, Bothell, WA, USA) was inserted into the ear artery and attached to a strain gauge transducer (Statham) via a tap so that arterial blood pressure and heart rate could be continuously recorded on a strip chart recorder (Statham) and to allow blood samples to be removed every hour for arterial blood gas analysis (Arieff *et al.* 1973). As previously described (Arieff *et al.* 1974*b*), paralysis was induced with succinylcholine (20 mg kg^{-1} I.V.) and, thereafter, the animal was mechanically ventilated, the ventilatory pump being adjusted to maintain arterial CO_2 pressure (P_{a,CO_2}) at about 35 mmHg. Supplementary doses of sodium pentobarbitone (5 mg kg^{-1} h^{-1} I.V.) were administered so that no transient rises in arterial blood pressure or heart rate occurred either spontaneously or in response to noxious stimulation of paw or ear.

In the twelve control rabbits mean (± S.D.) arterial blood pressure was 102 ± 6 mmHg and heart rate was 122 ± 6 beats min^{-1}. In the nine experimental rabbits, arterial blood pressure remained within the range 90–114 mmHg and the heart rate, between 110–135 beats min^{-1}.

Once stable anaesthesia and paralysis had been achieved, acute hypernatraemia was induced in the experimental animals by I.V. infusion of 1·7 M NaCl at a rate of 8·2 ml kg^{-1} over a 50 min period. A sustaining infusion of 0·32 M NaCl was then given for 1 or 4 h at 3 ml kg^{-1} h^{-1}, a rate calculated to maintain the plasma $[Na^+]$ between 170 and 190 mM (see Arieff, Guisado & Lazarowitz, 1977). Control animals received an infusion of 154 mM NaCl at the same rate and over the same time period as the experimental animals.

In control and experimental animals, the atlanto-occipital membrane was surgically exposed so that samples of cerebrospinal fluid (CSF) could be obtained from the cisterna magna by direct puncture of the atlanto-occipital membrane with a 25 gauge hypodermic needle. The CSF was used for measurement of osmolality, $[Na^+]$, $[K^+]$, $[Cl^-]$, pH and P_{CO_2} as previously described (Arieff *et al.* 1974*a*; Arieff, Kozniewska, Roberts, Vexler, Ayus & Kucharczyk, 1995).

Aseptic technique was maintained throughout the study to minimize the possibility of sepsis. In this or other groups described below, results from rabbits with severe hypotension (mean blood pressure below 50 mmHg), hypoxia (P_{O_2} below 45 mmHg), or hypercapnia (P_{CO_2} above 45 mmHg) were excluded from the analysis. At the end of these experiments, the rabbits were killed by overdose (200 mg I.V.) of sodium pentobarbitone. The top of the skull was removed using a trephine and rongeur and the brain removed for analysis (see below).

Group II: effects of chronic untreated hypernatraemia on brain adaptation

Chronic hypernatraemia was induced by gavage with hypertonic (1·7 M) NaCl (8·5 ml kg^{-1}) every other day for 1 week, and the rabbits were then allowed to drink 154 mM NaCl only (Arieff *et al.* 1977). It was estimated that this protocol would maintain plasma $[Na^+]$ at about 170 mM for 1 week. In practice, a blood sample was taken from the ear vein on days 2, 4, 6 and 7 for analysis of plasma $[Na^+]$, on the basis of which suitable adjustments were made to the volume of NaCl given the following day. Over this period, the rabbits were also monitored three times per day to assess their general status. They appeared lethargic, but showed no other signs of discomfort. At the end of the week the animals were anaesthetized, paralysed and artificially ventilated as described for Group I. They were then killed by overdose (200 mg I.V.) of sodium pentobarbitone and the brain was removed for analysis.

Group III: effects of treatment of acute and chronic hypernatraemia on brain adaptation

The treatment protocol was carried out on rabbits in which acute (as described for Group I) and chronic (as described for Group II) hypernatraemia had been induced. The acutely hypernatraemic rabbits remained anaesthetized, paralysed and artificially

ventilated throughout (see above). The chronically hypernatraemic animals, whose plasma [Na^+] had been maintained at 170 mM for 1 week (see above), were anaesthetized, paralysed and ventilated as for Group I. These animals underwent minor surgery for insertion of arterial and venous catheters and to allow samples of CSF to be taken as described for Group I. Both subgroups (acute and chronic hypernatraemia) then received sterile 77 mM NaCl as an I.V. infusion at rates chosen to bring the plasma [Na^+] to normal (below 150 mM) over specific time periods. Thus, the group with acute hypernatraemia received an infusion at 74 ml h^{-1} to reduce plasma [Na^+] to normal values over 4 h. In rabbits with chronic hypernatraemia, plasma [Na^+] was reduced to normal values over 4, 8 or 24 h by infusion at 54, 26 or 9 ml h^{-1}, respectively. Arterial blood samples were removed for analysis of blood gases and plasma values of [Na^+], [K^+], osmolality and [glucose]. For the purpose of constantly monitoring the rabbits, these measurements were made every hour in the groups treated for 4 or 8 h, and every 4 h in the group in which plasma [Na^+] was lowered to normal over 24 h. Minor adjustments were then made to the infusion rates if required. The data presented are the values at the end of the treatment periods. At the end of the experiments, samples of CSF for analysis were taken, the animals were then killed by overdose (200 mg I.V.) of sodium pentobarbitone and the brain was removed as described for Group I.

Analysis of brain composition

A thin slice of frontal cortex from each brain frontal hemisphere was removed with a scalpel and immediately frozen in liquid nitrogen. This was used to make a profile of amino acids using a Durham Microbore amino acid analyser (Beckmann Instruments) (Benson, 1977). Because the amino acid profile has been shown to change rapidly following death, care was taken to exclude from the study any rabbit which stopped breathing before the brain was removed. The grey matter was homogenized with 0·4 M sulphosalicylic acid containing a known quantity of L-norleucine, which served as an internal standard. The samples were centrifuged at 20000 *g* for 20 min and the supernatant injected into the amino acid analyser. We measured total amino acid concentration, and the individual concentrations of taurine, aspartic acid, serine, asparagine, glutamic acid, glutamine, glycine, alanine, arginine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine and lysine.

The remaining brain hemisphere tissue (excluding brainstem amd cerebellum) was placed in a flask filled with liquid nitrogen. The frozen tissue was pulverized to a coarse powder and samples were used for measurement of brain osmolality, [Na^+], [K^+] and water content (Arieff *et al.* 1973). Brain osmolality was measured by extraction of liquid nitrogen-frozen brain tissue in boiling distilled water (Arieff *et al.* 1972). Brain tissue water content was determined by taking the ratio (dry : wet) of the fresh brain tissue mass after and before oven drying at 100 °C for 24 h (Arieff *et al.* 1973). Brain [Na^+], [K^+] and [Cl^-] were measured after extraction of brain tissue in 0·75 M HNO_3 (Arieff *et al.* 1973).

Extracellular [Na^+], [K^+] and [Cl^-], osmolality, pH and P_{CO_2} were measured from the CSF. Brain extracellular fluid (ECF) space was determined as the endogenous Cl^- space relative to CSF, using a correction for the intracellular [Cl^-] as previously described (Katzman & Pappius, 1973). Brain intracellular water content, [Na^+] and [K^+] were calculated relative to the concentrations in CSF, using the corrected Cl^- ECF space as the extracellular space. Thus, the percentage of water in the brain was calculated from the dry : wet ratio (see above). Brain tissue [Na^+] and [K^+] values were converted to mmol (kg H_2O)$^{-1}$ by dividing the value in mmol (kg wet wt)$^{-1}$ by (percentage of water/100). Intracellular [Na^+] was calculated from these values as follows:

$$[Na^+]_{brain} = \frac{([Na^+]_{CSF} \times \%\,ECF) + ((100 - \%\,ECF) \times [Na^+]_i)}{100},$$

where $[Na^+]_{brain}$ (in mmol (kg H_2O)$^{-1}$), $[Na^+]_{CSF}$ (in mmol (kg H_2O)$^{-1}$) and $[Na^+]_i$ (in mmol (kg intracellular H_2O)$^{-1}$) are whole brain tissue, CSF and intracellular [Na^+] values, respectively, and % ECF space is the percentage endogenous Cl^- space with respect to the cisternal cerebrospinal fluid corrected for intracellular [Cl^-] (Katzman & Pappius, 1973). Intracellular [K^+] was calculated by substituting K^+ values for Na^+ values in this equation. The percentage of intracellular water was calculated by substituting H_2O for Na^+ and adjusting the units to those used for water, i.e.

$$(H_2O)_{brain} = \frac{((H_2O)_{CSF} \times \%\,ECF) + ((100 - \%\,ECF) \times (H_2O)_i)}{100},$$

where $(H_2O)_{brain}$ (in ml (100 g wet wt)$^{-1}$), $(H_2O)_{CSF}$ (in ml (100 g CSF)$^{-1}$) and $(H_2O)_i$ (in ml (100 g intracellular wet wt)$^{-1}$) are whole brain tissue, CSF and intracellular water contents, respectively. To calculate intracellular water content in (ml H_2O)(100 g intracellular dry wt)$^{-1}$ the percentage of intracellular water was divided by the percentage of intracellular dry weight (100 − percentage intracellular H_2O) × 100. The brain concentration of idiogenic osmoles was calculated by subtracting the brain [$Na^+ + K^+$ + amino acids] (in mmol (kg brain H_2O)$^{-1}$) from the measured brain osmolality, i.e. it was assumed that the osmotic coefficient for Na^+, K^+ and amino acids was one.

Studies in rats

The rat study protocol was approved by the Animal Research Committee (Baylor College of Medicine, Houston, TX, USA). The effects of untreated acute hypernatraemia on neurological status, brain histology and brain water content were studied. Rats were used because of our substantial experience with brain histology in rats with electrolyte disorders (Ayus, Krothapalli & Armstrong, 1985; Ayus, Krothapalli, Armstrong & Norton, 1989).

Male Sprague–Dawley rats (starting weight, 225–350 g) were kept in individual metabolic cages (room temperature, 22 °C) so that individual body weight could be monitored daily using standard procedures ((Heilig *et al.* 1989). Rats were allowed 7 days to adapt before initiation of the study. All rats had free access to food throughout the study. A detailed neurological examination (including evaluation of muscle tone and gait, and the response to sharp pinprick) was performed three times daily (early morning, noon and late afternoon) during experimental periods (see below). Blood was removed from the tail vein while rats were under light ether anaesthesia, and used for measurement of plasma [Na^+] as previously described (Arieff *et al.* 1973). Two experimental groups were studied at different levels of hypernatraemia.

Groups I and II: effect of untreated acute hypernatraemia on brain histology

On day 1, acute hypernatraemia was induced at two levels by two I.P. injections of 1 M NaCl (5 or 7·5 ml (100 g body wt)$^{-1}$, Group I or Group II, respectively) separated by an interval of *ca* 10 h. This procedure was calculated to increase plasma [Na^+] to 160–170 mM (Group I) or 180–190 mM (Group II), respectively. Plasma [Na^+] was measured before induction of hypernatraemia (on day 1), and 24 h after the first injection of hypertonic NaCl (on day 2). During this 24 h period, animals were allowed food and 154 mM NaCl, but no water, to drink.

Immediately after the second blood sample had been taken (at 24 h), all Group I animals were killed with an overdose (100 mg I.P.) of sodium pentobarbitone. The brains were then removed and processed for histological examination and measurement of water content (see below). Some of the Group II animals died within the 24 h observation period (see Results), no analysis was performed on these animals. Those that survived were allowed to eat and drink water *ad libitum*. Each was assessed according to a neurological score on a scale of 0–4: 0, no neurological dysfunction; 1–2, the rats were irritable and showed an exaggerated response to pinprick, but showed no seizure activity and could walk; 3–4, severe neurological dysfunction characterized by seizure activity, inability to walk and exaggerated startle reflex. Those rats with a score of 3–4 were killed. The rationale for this approach is that in our previous work (Ayus *et al.* 1985; Ayus *et al.* 1989), we found a close relationship between severe neurological dysfunction and changes in brain histology. In such cases, the animal was anaesthetized with ether, a sample of blood was removed for analysis of plasma [Na^+] and an overdose of sodium pentobarbitone (100 mg I.P.) was given. Except those which died prematurely, all animals in Group II were killed between days 3 and 7 (see Results). The brains were processed for histological examination and measurement of water content (see below).

Group III: control animals

Two 5 ml I.P. injections of 154 mM NaCl were given to control animals on day 1. After 24 h and again at the end of 1 month of observation, they were anaesthetized with ether and a blood sample taken. After the second blood sample was taken the rats were killed with an overdose (100 mg I.P.) of sodium pentobarbitone and the brains submitted for histological examination and measurement of water content (see below).

Histological examination of the brain and measurement of brain water content

Immediately after the animals had been killed, the skull was opened and the brain removed after severing it from the spinal cord at the upper cervical level. The brain was bisected with a sharp scalpel and one-half of the brain and brainstem were immersed in 10% buffered formaldehyde and allowed to fix for 1 week. The fixed half was then cut coronally into blocks at 3 mm intervals, dehydrated, and embedded in paraffin. Two sections 5 μm in thickness were cut from each block; one was stained with Haematoxylin and Eosin and the other with Haematoxylin and Eosin–Luxol Fast Blue stain (for myelin) (Sheehan & Hrapchak, 1980). The sections were examined under light microscopy and interpreted by a pathologist (D.L.A.) who knew nothing of the experimental conditions until all the results had been tabulated. The other half of the brain was used for measurement of water content as previously described (Vexler, Ayus, Roberts, Kucharczyk, Fraser & Arieff, 1994).

Data presentation and analysis

The data from both rabbits and rats in the text and tables are presented as means ± S.D. In the figures, data are presented as means ± S.E.M., n is the number of animals in each group. Significance was determined using analysis of variance (ANOVA), performed on a Macintosh Quadra 650 computer using Statview software (version 4.0; Abacus Concepts, Berkeley, CA, USA). Values of $P < 0.05$ were taken as significant.

RESULTS

Studies in rabbits

Group I: effects of acute untreated hypernatraemia on brain adaptation

In Group I rabbits, plasma [Na^+] was increased from 146 ± 3 to 179 ± 8 mM after 1 h ($n = 9$) and to 182 ± 5 mM after 4 h ($n = 9$) (Table 1). The corresponding brain osmolalities are shown in Fig. 1. The increases in brain osmolality were accompanied by significant decreases in both whole brain tissue and intracellular water content (Fig. 1). The whole brain tissue water content after 1 and 4 h of hypernatraemia was 352 ± 14 and 339 ± 11 ml (100 g dry wt)$^{-1}$, respectively. Both values were significantly decreased compared with control (371 ± 19 ml (100 g dry wt)$^{-1}$, $P < 0.01$). Most of the increase in brain osmolality was due to increases in brain intracellular [$Na^+ + K^+$] (Fig. 2). The whole brain tissue [Na^+] after 1 and 4 h of hypernatraemia was 292 ± 6 and 321 ± 19 mmol (kg dry wt)$^{-1}$, respectively. Both values were significantly increased compared with control (268 ± 9 mmol (kg dry wt)$^{-1}$, $P < 0.01$). The remainder of the increase in brain osmolality was due to significant increases in the total brain concentration of amino acids ($P < 0.01$; Table 2) and undetermined solute (idiogenic osmoles) ($P < 0.001$; Fig. 2). Thus, in rabbits with acute hypernatraemia for 1 or 4 h, the brain showed a significant loss of water, which appeared primarily to be intracellular (Fig. 1). The brain adapted with a corresponding increase in the intracellular concentrations of ($Na^+ + K^+$), idiogenic osmoles (Fig. 2) and amino acids (Table 2).

Group II: effects of chronic untreated hypernatraemia on brain adaptation

In Group II rabbits ($n = 8$), the plasma [Na^+] was increased to 170 ± 15 mM and maintained for 1 week (Table 1). The corresponding brain osmolality is shown in Fig. 1. Despite the increase in brain osmolality, brain intracellular water content had returned to normal values ($P = 0.7$), while the brain osmolality remained significantly increased compared with control ($P < 0.001$; Fig. 1). In contrast to the findings in acute hypernatraemia, the brain intracellular [$Na^+ + K^+$], while remaining significantly higher than control ($P < 0.001$), was significantly less than that after 1 or 4 h of acute hypernatraemia ($P < 0.005$; Fig. 2). The remainder of the increase in brain osmolality was due to significant increases in the brain concentration of amino acids ($P < 0.01$; Table 2) and idiogenic osmoles ($P < 0.001$; Fig. 2). Thus, in rabbits with chronic (1 week) hypernatraemia, the brain intracellular water content was normal (Fig. 1) and the brain had adapted with significant increases in the brain concentration of idiogenic osmoles, amino acids and ($Na^+ + K^+$).

Table 1. Plasma and arterial blood parameters measured in acute and chronic hypernatraemic rabbits

	Plasma				Arterial blood		
	$[Na^+]$ (mM)	$[K^+]$ (mM)	Osmolality (mosmol $(kg\ H_2O)^{-1}$)	[Glucose] (mM)	pH	P_{O_2} (mmHg)	P_{CO_2} (mmHg)
Control	146 ± 3	4·4 ± 0·6	304 ± 4	3·1 ± 0·3	7·44 ± 0·05	70 ± 9	33 ± 3
Acute hypernatraemia							
For 1 h	179 ± 8	3·8 ± 0·6	369 ± 14	6·4 ± 0·6	7·28 ± 0·07	69 ± 10	30 ± 7
For 4 h	182 ± 5	4·8 ± 0·5	390 ± 18	16·2 ± 2·1	7·21 ± 0·06	64 ± 15	34 ± 4
Treated over 4 h	146 ± 6	4·1 ± 1·2	308 ± 9	12·3 ± 2·6	7·28 ± 0·13	64 ± 7	39 ± 8
Chronic hypernatraemia							
For 1 week	170 ± 15	3·3 ± 0·5	364 ± 29	17·1 ± 1·8	7·36 ± 0·09	78 ± 18	33 ± 8
Treated over 4 h	143 ± 4	3·4 ± 0·7	307 ± 9	16·6 ± 1·8	7·24 ± 0·12	73 ± 6	37 ± 4
Treated over 8 h	144 ± 4	4·2 ± 0·9	319 ± 21	9·6 ± 3·1	7·26 ± 0·14	74 ± 7	36 ± 5
Treated over 24 h	146 ± 6	4·3 ± 1·3	312 ± 13	9·1 ± 5·5	7·29 ± 0·11	69 ± 8	38 ± 7

Values given as means ± S.D.

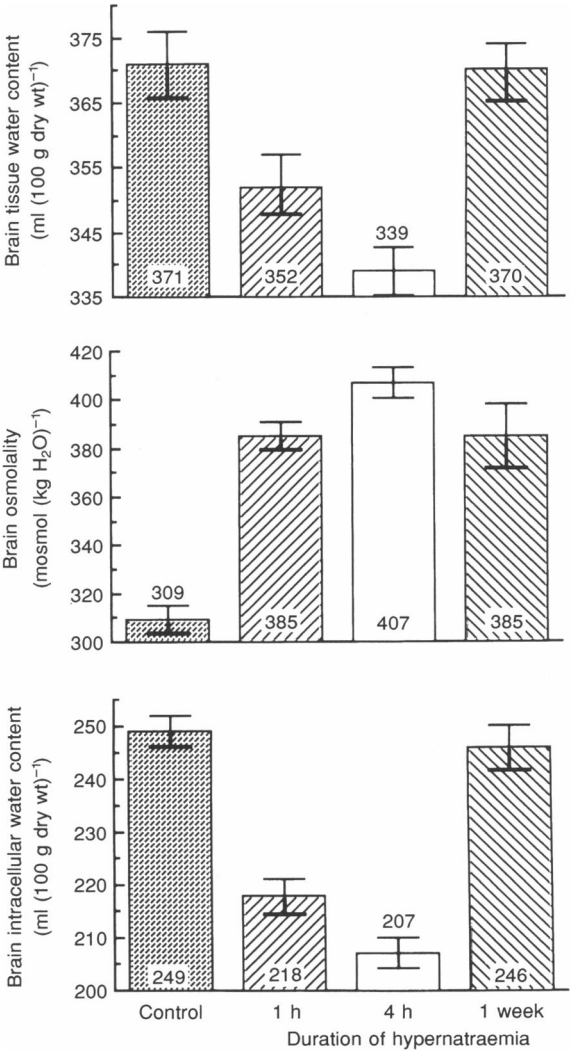

Figure 1. Brain tissue water content, osmolality and intracellular water content in hypernatraemic rabbits

The upper panel shows the brain tissue water content in control rabbits and rabbits with hypernatraemia for 1 h, 4 h and 1 week. The values after hypernatraemia for 1 or 4 h are significantly less than both the control and 1 week hypernatraemia values ($P < 0·01$), while the value after 1 week hypernatraenia is not significantly different from control. The middle panel shows brain osmolality. All hypernatraemic values are significantly greater than the control value ($P < 0·01$). The lower panel shows the corresponding brain intracellular water content. The values after hypernatraemia for 1 or 4 h are significantly less than both the control and 1 week hypernatraemia values ($P < 0·01$), while the value after 1 week is not significantly different from control.

Table 2. Cerebrospinal fluid and brain parameters measured in acute and chronic hypernatraemic rabbits

	Cerebrospinal fluid					Brain	
	Osmolality (mosmol (kg H_2O)$^{-1}$)	[Na^+] (mM)	[K^+] (mM)	pH	P_{CO_2} (mmHg)	ECF (g (100 g wet wt)$^{-1}$)	Total amino acid concentration (mmol (kg H_2O)$^{-1}$)
Control	309 ± 5	155 ± 5	3·6 ± 0·6	7·40 ± 0·08	42 ± 8	20·2 ± 2·2	33·5 ± 5·2
Acute hypernatraemia							
For 1 h	374 ± 8	187 ± 8	4·7 ± 1·2	7·36 ± 0·06	37 ± 6	24·3 ± 1·1*	39·8 ± 7·3
For 4 h	401 ± 12	196 ± 10	4·6 ± 0·2	7·35 ± 0·05	38 ± 5	24·4 ± 1·6*	51·7 ± 6·2
Treated over 4 h	319 ± 10	154 ± 7	3·6 ± 0·6	7·35 ± 0·07	45 ± 4	21·2 ± 0·9	38·2 ± 5·6
Chronic hypernatraemia							
For 1 week	376 ± 11	177 ± 14	3·7 ± 0·5	7·33 ± 0·06	39 ± 8	20·6 ± 1·3	53·4 ± 4·6
Treated over 4 h	319 ± 9	152 ± 8	3·5 ± 0·5	7·31 ± 0·06	43 ± 6	20·4 ± 0·7	51·7 ± 6·2
Treated over 8 h	325 ± 12	154 ± 7	3·6 ± 0·3	7·32 ± 0·07	41 ± 6	20·5 ± 0·9	40·6 ± 5·3
Treated over 24 h	322 ± 11	153 ± 8	3·4 ± 0·4	7·33 ± 0·06	43 ± 5	19·7 ± 1·1	39·8 ± 4·7

ECF, extracellular fluid. Values given as means ± S.D. * Significantly different from control, $P < 0·01$.

Group III: effects of treatment of acute and chronic hypernatraemia on brain adaptation

Previous studies in hypernatraemic humans suggest that if plasma [Na^+] is lowered to normal values in less than 4 h, fatal cerebral oedema can occur (Lutrell & Finberg, 1959; Smith, Block, Arieff, Blumenkrantz & Coburn, 1974), while therapy over 24 h does not usually lead to cerebral oedema (Banister, Siddiqi & Hatcher, 1975; Bowling & Bourn, 1978). Thus, we lowered plasma [Na^+] in the hypernatraemic rabbits to normal values over intervals of 4–24 h. In rabbits with acute (4 h) hypernatraemia ($n = 7$), the plasma [Na^+] was lowered from 182 ± 5 to 146 ± 6 mM ($n = 7$) over 4 h (Table 1). Brain herniation was not observed. The brain intracellular water content was 260 ± 25 ml (100 g intracellular dry wt)$^{-1}$, significantly greater than that after either 4 h of acute hypernatraemia (207 ± 8 ml (100 g intracellular dry wt)$^{-1}$; $P < 0·001$) and control (249 ± 13 ml (100 g intracellular dry wt)$^{-1}$; $P < 0·005$). The whole brain tissue water content was 383 ± 33 ml (100 g dry wt)$^{-1}$, significantly greater than after 4 h hypernatraemia (339 ± 11 ml (100 g dry wt)$^{-1}$; $P < 0·01$). Brain osmolality decreased significantly to 336 ± 16 mosmol (kg H_2O)$^{-1}$ ($P < 0·01$) but remained significantly above control ($P < 0·01$). In terms of brain solute, the brain [$Na^+ + K^+$] decreased significantly compared with the value after 4 h of hypernatraemia (227 ± 7 *vs.* 167 ± 16 mmol (kg intracellular H_2O)$^{-1}$, $P < 0·001$) with therapy, while brain amino acid

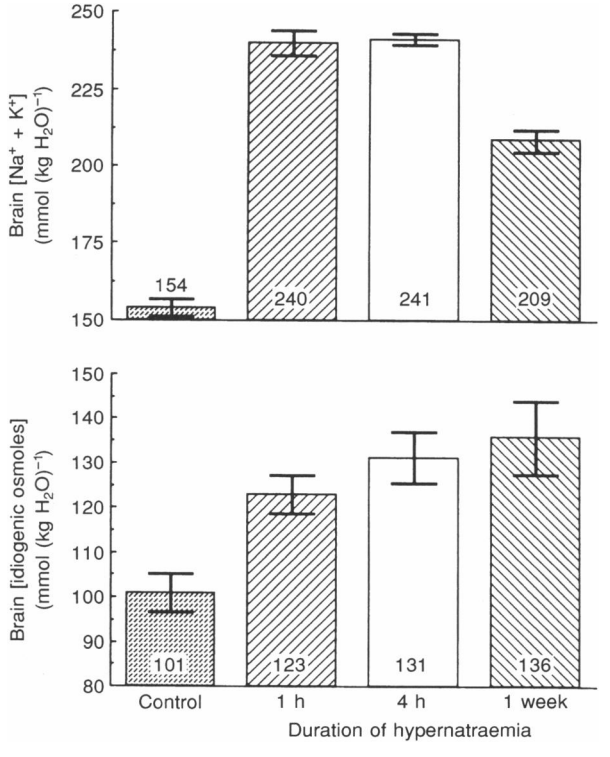

Figure 2. Brain intracellular concentrations of ($Na^+ + K^+$) and idiogenic osmoles in hypernatraemic rabbits

The upper panel shows the brain intracellular [$Na^+ + K^+$] in control rabbits and in rabbits with hypernatraemia for 1 h, 4 h and 1 week. All hypernatraemic values are significantly greater than control ($P < 0·01$). The lower panel shows the corresponding values for brain idiogenic osmole concentration. The values after hypernatraemia for 1 h, 4 h or 1 week are significantly greater than control ($P < 0·01$).

Table 3. Individual brain amino acid concentrations (in mmol (kg H_2O)$^{-1}$) in rabbits with hypernatraemia for 1 h, 4 h and 1 week

	Asp	Glu	Gln	Gly	Tau	Ser	GABA	Phos
Control	3·4 ± 0·07	6·4 ± 1·9	3·6 ± 0·7	2·0 ± 0·6	2·9 ± 0·3	1·9 ± 0·3	2·65 ± 0·9	2·9 ± 0·1
Acute hypernatraemia								
For 1 h	3·0 ± 0·6	6·4 ± 2·1	6·4 ± 2·1	4·5 ± 2·1	2·4 ± 0·6	0·9 ± 0·2	2·4 ± 0·9	2·8 ± 1·3
For 4 h	3·6 ± 1·8	7·4 ± 2·3	5·6 ± 1·7	4·2 ± 1·5	2·6 ± 0·7	—	2·5 ± 0·8	2·7 ± 0·8
Chronic hypernatraemia								
For 1 week	3·4 ± 1	13·4 ± 1·3	11·1 ± 3·5	3·7 ± 0·6	3·2 ± 1·2	—	1·7 ± 0·4	2·2 ± 0·8

Abbreviations: Asp, aspartic acid; Glu, glutamic acid; Gln, glutamine; Gly, glycine; Tau, taurine; Ser, serine; Phos, phosphoethanolamine. Concentrations of asparagine, alanine, arginine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine and lysine were <1 mmol (kg H_2O)$^{-1}$.

(38·2 ± 5·6 mmol (kg H_2O)$^{-1}$) and idiogenic osmole (128 ± 20 mmol (kg H_2O)$^{-1}$) concentrations remained unchanged ($P > 0·1$). Thus, therapy of acute hypernatraemia over 4 h resulted in significant brain oedema despite a significant decrease in brain intracellular [$Na^+ + K^+$], because the brain concentration of amino acids and idiogenic osmoles failed to decrease.

Rabbits with chronic (1 week) hypernatraemia were treated over periods of 4 ($n = 8$), 8 ($n = 12$) or 24 h ($n = 8$), with plasma [Na^+] decreased below 150 mM (Table 1). Brain herniation was not observed. The brain intracellular water content after therapy for either 8 or 24 h was significantly greater ($P < 0·05$) than that for both controls and untreated chronic hypernatraemia (Fig. 3). The whole brain tissue water content after therapy of chronic hypernatraemia for either 8 (386 ± 30 ml (100 g dry wt)$^{-1}$) or 24 h (391 ± 29 ml (100 g dry wt)$^{-1}$) was also significantly greater than either the control value (371 ± 19 ml (100 g dry wt)$^{-1}$), or the value after 1 week of chronic hypernatraemia (370 ± 16 ml (100 g dry wt)$^{-1}$) ($P < 0·01$). These changes in brain water content were accompanied by significant decrements of brain osmolality compared with untreated chronic hypernatraemia ($P < 0·005$), but all remained significantly above control values ($P < 0·05$; Fig. 3). In terms of brain solute, the intracellular [$Na^+ + K^+$] decreased significantly after 4, 8 or 24 h of therapy compared with the value in untreated chronic hypernatraemia ($P < 0·001$; Fig. 4), while brain amino acid (Table 2) and idiogenic osmoles (Fig. 4) concentrations remained unchanged ($P > 0·1$). Thus, therapy of chronic hypernatraemia (over 4, 8 or 24 h) resulted in significant brain oedema despite a significant decrease in brain intracellular [$Na^+ + K^+$], because the brain amino acid and idiogenic osmole concentrations failed to decrease. The brain oedema was significant, whether determined as intracellular or whole brain tissue water content, compared with control values or those after 1 week of hypernatraemia.

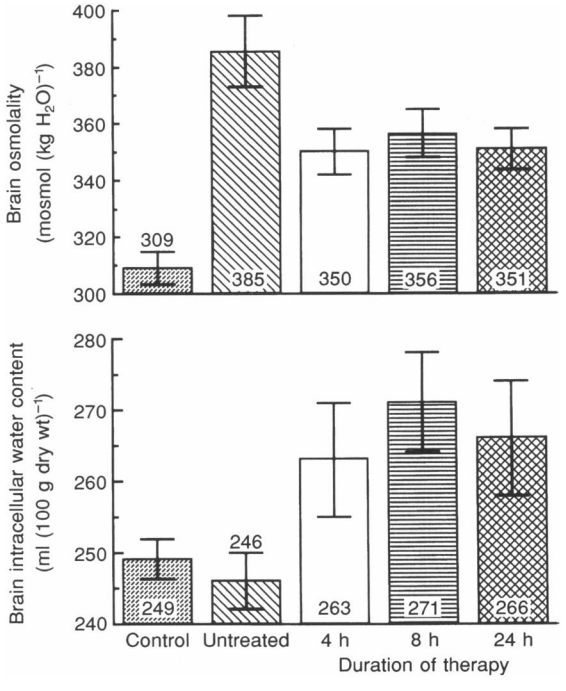

Figure 3. Brain osmolality and intracellular water content in rabbits before and after treatment for hypernatraemia

The upper panel shows the brain osmolality in control rabbits, rabbits with untreated chronic (1 week) hypernatraemia, and rabbits in which hypernatraemia was lowered to normal values over 4, 8 or 24 h. All values in rabbits with treated hypernatraemia are significantly less than the value in rabbits with chronic hypernatraemia ($P < 0·01$) and significantly greater than control ($P < 0·01$). The lower panel shows the corresponding values for brain intracellular water content. The values after therapy over 4, 8 or 24 h are significantly greater than both the control and 1 week hypernatraemia values ($P < 0·01$).

Brain amino acid concentration

Total brain amino acid concentrations are shown in Table 2. Because this study was directed at evaluation of the possible contribution of amino acids to brain osmotic activity, amino acid values below ~1 mmol (kg H_2O)$^{-1}$ are not reported. Individual brain amino acid concentrations are shown in Table 3. After therapy for 4, 8 or 24 h there were no significant differences. Thus, during hypernatraemia, brain amino acid concentrations reached a peak after 4 h, and did not undergo important changes during 1 week of hypernatraemia. During therapy for hypernatraemia, the failure of amino acids (Table 2) and idiogenic osmoles (Fig. 4) to dissipate appeared to play an important role in the brain oedema.

Rat studies

Groups I and II: effects of untreated acute hypernatraemia on brain histology

Group I. In Group I rats ($n = 25$), the plasma [Na^+] was increased from the control value of 143 ± 2 ($n = 25$) to 170 ± 4 mM after 24 h and there was no mortality during that time. However, these rats were irritable and showed exaggerated response to pinprick. No seizure activity or gait abnormalities were observed. All twenty-five rats were killed after 24 h hypernatraemia. There was no significant change in whole brain water content (352 ± 5 ml (100 g dry wt)$^{-1}$) compared with Group III controls (359 ± 8 ml (100 g dry wt)$^{-1}$; $P > 0.05$). The brain histology of Group I was normal, being comparable to that of Group III control animals.

Group II. Among Group II rats ($n = 25$), the plasma [Na^+] was increased from a control value of 141 ± 3 to 187 ± 3 mM over 24 h and the resulting mortality during that period was 44%. The remaining fourteen rats in this group were monitored and killed once a neurological score of 3–4 was reached (between days 3 and 7). Although food and water were freely available, none of the animals ate or drank, as determined by the metabolic balance studies. Consequently, there was substantial weight loss in these rats from 306 ± 20 to 250 ± 10 g. Plasma [Na^+] measured immediately before killing was 184 ± 2 mM ($n = 14$). The whole brain tissue water content of these rats ($n = 14$) was 337 ± 15 ml (100 g dry wt)$^{-1}$, which was significantly less than that in both the Group III controls (359 ± 8 ml (100 g dry wt)$^{-1}$, $P < 0.01$) and the Group I 24 h hypernatraemic rats (352 ± 5 ml (100 g dry wt)$^{-1}$, $P < 0.01$).

All brain histology for Group II rats showed alteration from that expected normally (Fig. 5*A* and *B*). Not every animal had the same lesions. In this study, dark neuronal change was not counted as abnormal, because the brains were prepared by immersion fixation. However, spongy change was considered to be abnormal and was identified when there was a loosening of the neutrophil, giving a cystic appearance on Haematoxylin and Eosin staining. This was mostly apparent in myelinated tracts, but also occurred in the basal ganglia and brainstem. Where tracts and nuclei were intermixed, spongy change was not observed by light microscopy.

Cellular lesions were classified as early (no macrophages) or advanced (macrophages present). Early lesions occurred mostly in nuclear areas (grey matter) and consisted of karyorrhexis in cells considered to be neurons. An occasional endothelial cell also showed karyorrhexis. These early lesions were also sometimes associated with a spongy change, and with prominent vessels, but the spongy change was sometimes seen as an isolated finding. When this occurred in

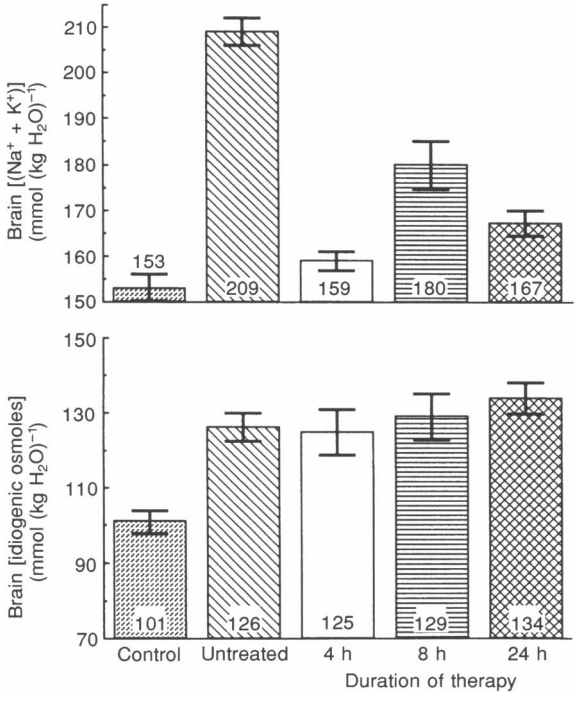

Figure 4. Brain intracellular concentrations of (Na^+ + K^+) and idiogenic osmoles in rabbits before and after treatment

The upper panel shows the brain intracellular [$Na^+ + K^+$] in control rabbits, rabbits with untreated chronic (1 week) hypernatraemia, and rabbits in which hypernatraemia was lowered to normal values over 4, 8 or 24 h. All values in rabbits with treated hypernatraemia are significantly less than the value in rabbits with untreated chronic hypernatraemia ($P < 0.01$). The lower panel shows the corresponding values for brain idiogenic osmole concentration. The values after therapy over 4, 8 or 24 h are significantly greater than control ($P < 0.001$) and not significantly different from the value after 1 week of hypernatraemia ($P > 0.3$).

the white matter, the change involved the myelin which appeared distorted on myelin stains. The advanced lesions were hypercellular, and consisted of karyorrhectic nuclei, as well as macrophages, proliferating endothelial cells, and an occasional astrocyte. Dead cells and swollen axons were present in some of the lesions. The white matter near the advanced lesions appeared microcytic in several cases, and there was loss of myelin in these areas. In three rats with a neurological score of 4, eosinophilic change in neurons of the hippocampus was identified, suggesting hypoxic–ischaemic injury of recent onset. Distribution of brain lesions in the different brain regions of a total of fourteen rats was: cerebral cortex, 5 rats; subcortical white matter, 8 rats; hippocampus, 7 rats; thalamus, 11 rats; basal ganglia, 6 rats; cerebellum, 8 rats; brainstem, 9 rats. The cerebellar lesions were usually mid-line in the cerebellar vermis (Fig. 5*A* and *B*), were focal within the folium and involved internal granular cell layer neurons and the adjacent white matter. The Purkinje cells were preserved.

DISCUSSION

The present study demonstrates that in rabbits with acute or chronic hypernatraemia, brain volume regulation is achieved by a combination of increased intracellular concentrations of osmotically active cations ($Na^+ + K^+$) and *de novo* generation of osmotically active solutes (idiogenic osmoles and amino acids) (Figs 6 and 7). This brain osmolality increase would presumably serve to protect the brain from the more severe dehydration that would be experienced without it. For example, after 1 and 4 h of acute hypernatraemia in rabbits, brain intracellular water content was decreased by 12 and 17%, respectively. These findings are quantitatively very similar to results previously reported in acutely hypernatraemic kittens (Finberg *et al.* 1959). If there had been no increase in brain amino acid concentration and no generation of idiogenic osmoles, brain water content would theoretically have decreased by 32%, a level that is incompatible with survival (Finberg *et al.* 1959). No further increase in brain

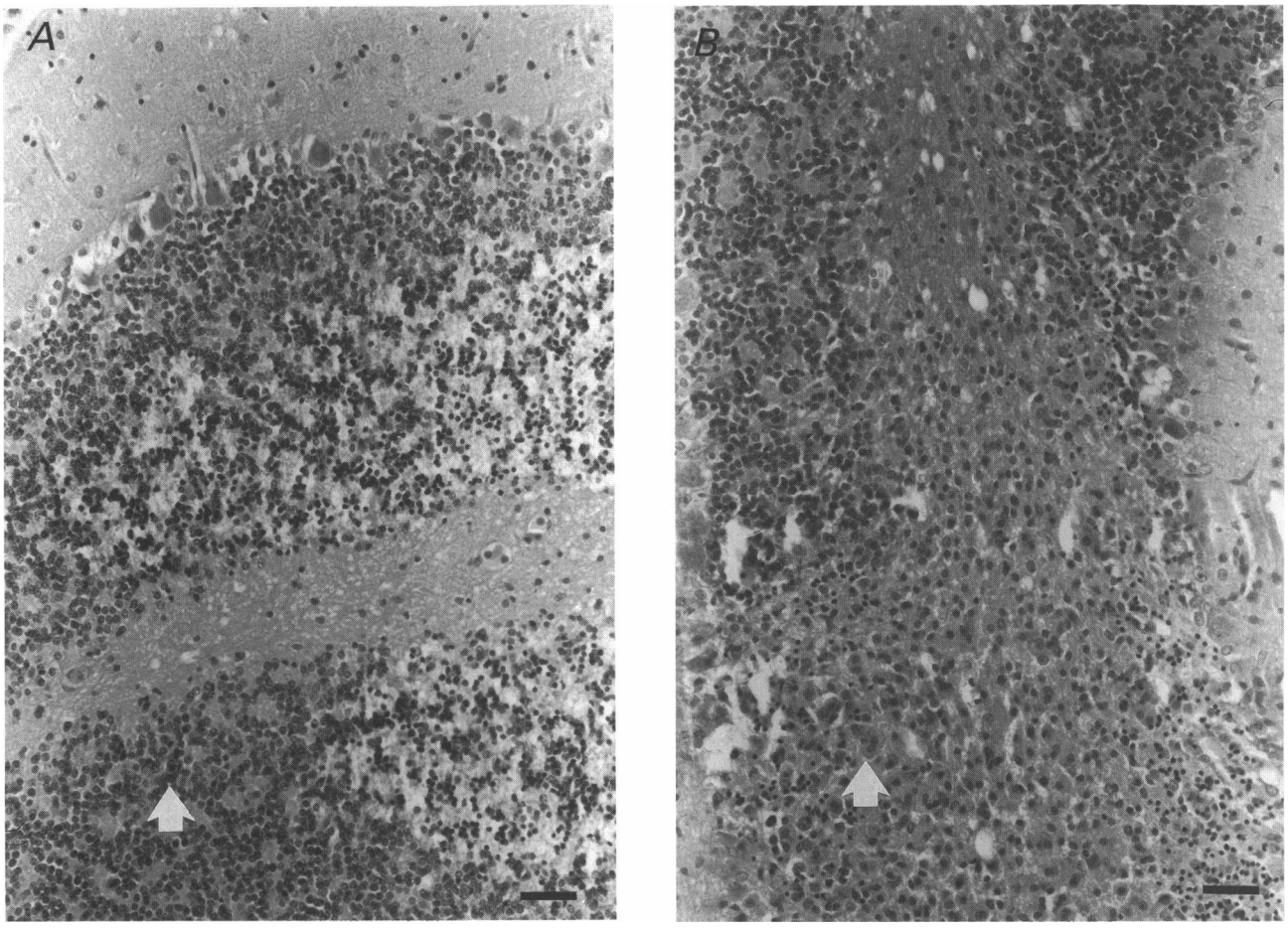

Figure 5. Brain sections of rats with acute untreated hypernatraemia (plasma [Na^+], 187 ± 3 mM)
A, the cerebellum showing early spongy change (arrow) in portions of the internal granular cell layer (Haematoxylin and Eosin; scale bar, 250 μm). *B*, the same region of cerebellum as shown in *A* (Haematoxylin and Eosin; scale bar, 62·5 μm) with a more advanced proliferating lesion (arrow) which involves internal granular cell layers (grey matter) and intervening white matter. There are increased numbers of cells (macrophages) and vessels.

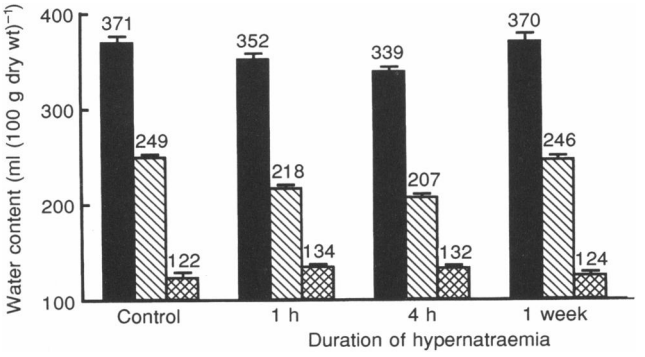

Figure 6. Measured brain tissue water content (■) and calculated intra- (▨) and extracellular (▩) water contents in controls and in rabbits with hypernatraemia induced for 1 h, 4 h and 1 week

With hypernatraemia for 1 or 4 h there was a loss of tissue water due to decrease in intracelluar water. There was a small gain in extracellular fluid as water left the intracellular space. After 1 week of hypernatraemia, neither the tissue, intracellular nor extracellular water were significantly different from control values ($P > 0{\cdot}05$).

osmolality was observed after 1 week of chronic hypernatraemia. This is consistent with our previous finding that an increase in idiogenic osmoles is more important in brain volume homeostasis in chronic, rather than in acute hypernatraemia (Arieff *et al.* 1977).

In the previous studies, we had evaluated brain amino acids as α-amino nitrogen, which also includes urea and other compounds that are not amino acids (Arieff *et al.* 1977), whereas in the current study, we measured amino acids specifically (Benson, 1977). Our evaluation of amino acids in hypernatraemic brain is similar to that reported by others (Lockwood, 1975; Heilig *et al.* 1989; Lien *et al.* 1990). In addition, we had previously included Cl^- as an osmotically active brain intracellular solute, but it is now known that most brain Cl^- is extracellular (Katzman & Pappius, 1973; Cserr, DePasquale, Nicholson, Patlak, Pettigrew & Rice, 1991). Thus, brain Cl^- should probably not be included in estimations of brain intracellular osmotic activity and in the current study, we did not include it. For the purpose of theoretical calculation only, we assumed that the osmotic coefficient of amino acids, Na^+ and K^+ is unity. Thus, the increase in concentration of amino acids plus idiogenic osmoles in rabbits with acute hypernatraemia (1–4 h) accounted for 42–49 % of the total increase in brain

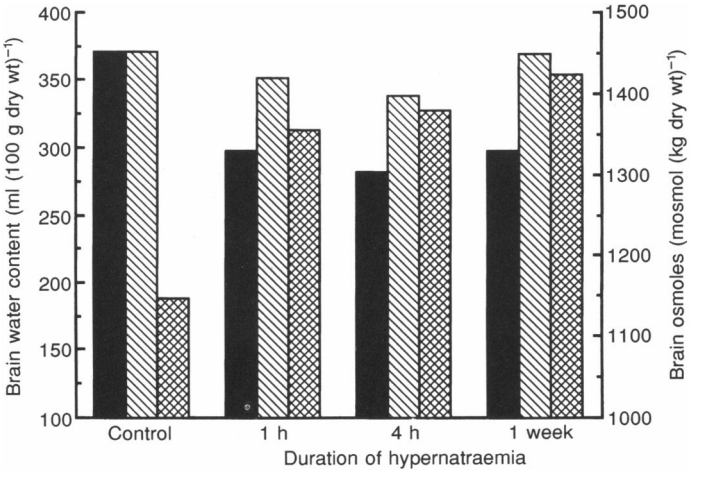

Figure 7. Measured brain tissue water content (▨) compared with the theoretical brain tissue water content (■) if the brain behaved as a perfect osmometer

For rabbits with hypernatraemia for 1 h, 4 h or 1 week, the measured brain water content was always higher than the theoretical value (left-hand ordinate axis). The brain osmole content (▩, right-hand ordinate axis) was significantly increased after only 1 h of hypernatraemia and remained significantly increased ($P < 0{\cdot}05$) during hypernatraemia of 4 h or 1 week duration.

osmolality. However, in rabbits with chronic hypernatraemia (1 week), the increase of amino acids plus idiogenic osmoles accounted for 71 % of the total increase in brain osmolality.

We made no attempt to determine the identity of the idiogenic osmoles present in the brains of rabbits with hypernatraemia. Studies by others suggest that such idiogenic osmoles include myoinositol, betaine, phosphocreatine and glycerophosphorylcholine (GPC), creatine, other methylamines, choline, sorbitol and other polyols (Heilig *et al.* 1989; Lien *et al.* 1990). Because brain osmolality was not measured in these studies, the possible osmotic contribution of these organic compounds was not deduced. Indeed the actual osmotic activity of the various brain solutes is still not known. In the present study, we assumed an osmotic activity of 1 mosmol $mmol^{-1}$. The signal for generation of these idiogenic osmoles is not known, but appears to be a function of hypernatraemia *per se*, since hyperosmolality induced with mannitol did not induce idiogenic osmole formation (Arieff *et al.* 1974*b*).

Estimates of brain intracellular water content and $[Na^+]$ were based on evaluation of the extracellular fluid (ECF) space as the Cl^- space corrected for brain intracellular chloride (Katzman & Pappius, 1973). There are potential problems with any methodology used to measure brain ECF, because of variation in the penetration of various markers, presence of different compartments and the sink action of the cerebrospinal fluid (Davson, 1968). The method we have employed in the present study yielded values very similar to those obtained with other chemical markers such as sulphate, thiocyanate, bromide, inulin or sucrose (Levin, Fenstermacher & Patlak, 1970; Katzman & Pappius, 1973). However, because of possible variation between methods, the interpretation of intracellular water content and $[Na^+]$ values is limited by the methodology employed. For this reason, we have provided measurements of both total brain $[Na^+]$ and water content, as well as estimates of intracellular values.

In contrast to our observations, a study of hypernatraemia in the rat (Cserr *et al.* 1991, in which plasma $[Na^+]$ was 188 mM) did not show a decrease in brain intracellular water content, even though there was a significant 7% decrease in total brain tissue water content a value similar to that observed in rabbits with acute hypernatraemia in the present study. Our measurements indicated that, in rabbits with acute hypernatraemia, the ECF space was significantly increased, while Cserr *et al.* (1991) reported that ECF space was significantly decreased. Methodological differences may account for these differences in brain ECF space and thereby in intracellular water content evaluation, there may also be species differences (rat *vs.* rabbit). The changes in intracellular $[Na^+]$ and $[K^+]$ reported in hypernatraemic rats (Cserr *et al.* 1991) were quantitatively similar to those observed in rabbits in the present study.

As indicated above, our data strongly suggest that generation of idiogenic osmoles and amino acids by the brain plays an important role in preservation of brain volume during hypernatraemia. By contrast, it seems that these amino acids and idiogenic osmoles are detrimental when the hypernatraemia is corrected to normal values. Thus, brain idiogenic osmoles and amino acids remained elevated when either acute or chronic hypernatraemia was corrected to normal values, even though brain intracellular $[Na^+ + K^+]$ was significantly decreased. Moreover, brain oedema was present both after correction of acute hypernatraemia to normal values over 4 h (brain water content, 260 ± 25 ml (100 g intracellular dry wt)$^{-1}$) and after correction of chronic hypernatraemia to normal values over 4, 8 or 24 h of therapy. Our data therefore indicate that after correction of either acute or chronic hypernatraemia brain oedema develops due to the failure of both amino acids and idiogenic osmoles to dissipate.

In rats with acute hypernatraemia, we found that both mortality and histological evidence of brain damage were related to the level of plasma $[Na^+]$. In Group I rats, in which the plasma $[Na^+]$ was increased to 170 ± 4 mM, there was no mortality, no histological evidence of brain damage and no change in brain water content. By contrast, in Group II rats in which plasma $[Na^+]$ was increased to 187 ± 3 mM ($P < 0.01$), mortality had occurred in 44% of the rats by 24 h, the rats exhibited both severe neurological dysfunction and histological changes in the brain. In Group II rats with acute hypernatraemia, it appeared that the deep mid-line nuclei of the cerebrum were the sites with the most pathology. The lesions were usually bilateral.

Thus, the present study demonstrates for the first time that in animals with experimental hypernatraemia myelinolytic brain lesions can be induced by elevation of plasma $[Na^+]$ from normal to hypernatraemic values. Similar lesions have been reported in hypernatraemic human subjects (Ayus *et al.* 1987), these were initially described over 30 years ago and were defined as myelinolysis (Adams, Victor & Mancall, 1959). Other authors have stressed that the lesions were not 'demyelination', but that there was an initial loss of myelin, that evolved into destruction of all the tissue elements, with resulting cavitation (Mathews & Moossy, 1975). The lesions observed in the present study did appear to progress through stages of spongy change in the myelin to individual cell death, with increased numbers of macrophages, prominent vessels, and, in some of the white matter lesions, microcystic changes. They did not excite an inflammatory response by leucocytes or lymphocytes. The lesions provoked in these animals by pure hypernatraemia are therefore similar to those which have been described in humans (Adams *et al.* 1959), in which concurrent pathological conditions preclude assessment of the role of hypernatraemia *per se*.

Thus, it seems likely that the results of the current study can be extrapolated to patients with hypernatraemia. With acute hypernatraemia, and the pathology seen in this rat model are similar to previously reported findings in hypernatraemic patients (Finberg, Kiley & Luttrell 1963; Simmons *et al.* 1974). Most important, however, this study indicates for the first time that the failure of undetermined solute in the brain to dissipate during therapy of hypernatraemia, both acute and chronic, results in significant brain oedema which can increase the already substantial morbidity and mortality associated with this condition.

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