4. In order to substantiate the assumption that the Nernst potential for K^+ reflects the magnitude of the cell membrane potential, the values of the Nernst K^+ potentials were compared with those of triphenylmethyl phosphonium bromide, (TPMP), a lipophilic cation. The mean values were: E_K 82±2 mV; E_{TPMP} 88±3 mV. These data are close to directly measured values of the cell membrane potential in perfused rectal glands, i.e., 78 mV (Walsh et al, Bulletin, MDIBL 20:121, 1980). E_{TPMP} was not affected by changes of external tonicity (hypotonic, i.e., urea-free saline; hypertonic salines), whereas depolarizing agents such as high external K^+ or ouabain also depressed the values of E_{TPMP}. Thus, it is justified to conclude that the cellular uptake of K^+ in urea-free salines, and the loss of K^+ in hypertonic salines (see 3 above) proceeds at a constant electrochemical gradient of tissue electrolytes.

The above data, i.e., absence of direct coupling between urea and K^+ fluxes; lack of cationic specificity; tonicity-dependent changes of cell water and electrolytes at a constant electrochemical gradient are consistent with the previously advanced hypothesis. This investigation was supported by grants from NIH (AM-12619) and the Whitehall Foundation.

CONTROL OF EXTRACELLULAR AND CELL VOLUMES IN BRAIN OF LITTLE SKATE (RAJA ERINACEA)

Helen F. Cserr, Ken Mackie, Eric Moody and Michael Bradbury, Department of Physiology, Brown University, Providence, R.I., and Department of Physiology, King's College, London, England

A considerable literature has accumulated on the control of cell volume in the face of osmotic disturbances. In the vertebrate brain the interstitial fluid is separated from blood by a tight endothelium or epithelium (the blood-brain barrier). Hence this extracellular fluid may also be subject to control by specific transport mechanisms. Indeed, in mammals there are big shifts of sodium and chloride in and out of brain, tending to maintain extracellular volume constant in hyper- and hypo-osmolar states respectively (reviewed by Bradbury, M.W.B., The Concept of a Blood-Brain Barrier, publ. Wiley: Chichester; Patel, P. & Cserr, H.F., In preparation).

Since the little skate, Raja erinacea, will tolerate considerable osmolality changes in its external environment and since its body fluids largely conform to these, shifts in the extracellular ion content in the brain of this species have been estimated - the eventual aim being to localize the site of these net fluxes (blood-brain, extradural fluid-brain or cerebrospinal fluid-brain) with radioactive isotopes. Free swimming fish were subjected to sea-water plus 65 mM NaCl (hypertonic - these were also injected with a Na Cl i-m to raise the osmolality of their body fluids by the same proportion as the sea-water) or to dilute sea-water (hypotonic - 50% of normal osmolality). At a set time, blood was sampled by cardiac puncture and after exanguination, the brain removed, frozen on dry ice and standard pieces of telencephalon and medulla removed into tared vials. The brain pieces were dried to constant weight and extracts of the dried tissues in 0.75 N HNO_3 analyzed for chloride coulometrically and for sodium and potassium by emission flame photometry.

In Table 1, is recorded brain-water, expressed as chloride and non-chloride spaces. The predicted changes in these spaces as ideal osmometers have been calculated with the plasma concentration of sodium and chloride as a standard of reference, since the other main osmotically active solute in plasma, urea, diffuses freely in and out of brain. Whilst the non-chloride component of brain-water behaved as a near perfect osmometer with respect to plasma [$Na^+ + Cl^-$], especially in hypotonic conditions, there was considerable regulation of the chloride space, this being near perfect in the hypertonic conditions. In Table 2, it can be seen that sodium moved in the same direction and in equivalent amounts to chloride, whereas potassium changes in brain were small and not significant. Analysis of telencephalon for ninhydrin positive substances indicated little change in the amount of amino acids in this part of brain under the conditions used. In contrast to telencephalon, the medulla showed little control of water and minimal ionic shifts.

TABLE 1. Observed and predicted chloride (extracellular) and non-chloride (cell) spaces in skate telencephalon

Condition	n	Chloride space, ml. g^{-1} dry wt.		Non-Cl-space, ml. g^{-1} dry wt.	
		Observed	Predicted	Observed	Predicted
Isotonic	7	1.45±0.06	-	3.26±0.09	-
Hypertonic 2 hr	5	1.52±0.03	1.23	2.89±0.10	2.77
Hypertonic 4 hr	5	1.40±0.03	1.20	2.89±0.05	2.69
Hypotonic 24 hr	6	1.62±0.03	1.79	3.95±0.08	4.02

Predicted spaces are calculated on supposition that they behave as perfect osmometers in relation to plasma [Na^+ + Cl']. Values are means ±SE.

TABLE 2. Chloride, sodium and potassium in skate telencephalon, mmol. $(100\ g)^{-1}$ dry wt., with % change in plasma [Na^+ + Cl']

Condition	n	Plasma, %Δ in [Na^+ + Cl']	Telencephalon		
			Chloride	Sodium	Potassium
Isotonic	7	-	38.5±0.8	42.2±0.8	55.3±0.3
Hypertonic 2 hr	5	+17.6	46.5±1.4	51.1±1.8	57.6±2.4
Hypertonic 4 hr	5	+21.2	45.5±2.2	49.9±1.9	58.7±1.8
Hypotonic 24 hr	6	-18.9	34.0±0.7	38.2±1.2	53.6±1.1

Values are means ±SE.

It is concluded that there is a rapid and considerable regulation of the extracellular fluid volume in skate telencephalon. Cell volume does not appear to be so well controlled over these relatively shoft time periods. Preliminary experiments with ^{22}Na suggest that the source of the sodium entry in hypertonic conditions is not extradural fluid.

H INTRACRANIAL PRESSURE DYNAMICS IN SKATES.

Eric Moody, Michael Bradbury and Helen Cserr, Division of Biology and Medicine, Brown University, Providence, R.I., and Department of Physiology, University of London King's College, London, England

In mammals, osmotic disturbances are associated with marked changes in intracranial pressure (ICP). The change in pressure is caused by the osmotic flow of water either into or out of the rigid cranial cavity. As part of a general study of the CNS response to osmotic disturbances in skates, Raja erinacea and Raja ocellata, we found that ICP failed to change in hypernatremia. In an attempt to explain this observation, we have analyzed factors related both to the initial perturbation (change in intracranial volume) and to compensatory mechanisms for alleviating changes in volume and pressure (compliance of the neural axis, resistance to fluid outflow).