Water Uptake and Energy Metabolism in Brain Slices from the Rat

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1. Exchange of $^3\text{H}_2\text{O}$ with H_2O takes place rapidly in incubated rat brain slices but at a lower rate in slices from infant brain than from adult brain. The temperature coefficient (Q_{10}) of the exchange process, between 37 and 4°C, is 1.76 with infant brain and 1.26 with adult brain. The exchange process is unaffected by the presence of ouabain or 2,4-dinitrophenol. 2. An approximately linear relationship exists between water uptake and the concentration of ATP in the incubated slices in the presence of various concentrations of glucose. Little or no change occurs in water uptake and ATP concentration in the presence of a glucose concentration exceeding 3 mM. A linear relationship also exists between the water uptake and ATP concentration in the presence of 10 mM-glucose and various concentrations of sodium L-glutamate but the line is parallel to that found with changed glucose concentrations and shifted in the direction of increased water uptake. A similar parallel relationship exists between water uptake and ATP concentration in the presence of 2,4-dinitrophenol, but the amount of water uptake is significantly smaller in the presence of 2,4-dinitrophenol than in its absence. 3. Copper chloride (0.3 mM) or mercuric chloride (0.3 mM) both increase water uptake and diminish the ATP concentration in slices. Sodium malonate (2 mM) or sodium D-glutamate (10 mM) has similar effects. 4. Substances, or conditions, affecting water uptake in incubated brain slices may be divided roughly into two classes in accordance with their effects on adenosine triphosphatase and membrane permeability, but there may be considerable lack of specificity.

The early demonstrations that brain slices incubated in a saline medium take up water (Elliott, 1946) and that brain respiration is an important factor in controlling water uptake (Stern, Eggleston, Hems & Krebs, 1949) were followed by numerous studies concerning the conditions governing water movement in isolated brain tissue. Leaf (1956) made it clear that the fluid entering the tissue is not water alone but a solution approximately iso-osmotic with the medium and that the dependence of fluid exchange on tissue metabolism is due to the requirement of metabolic energy for the maintenance of ionic gradients. The quantity of Na^+ entering the tissue may be accounted for partly by the influx of sodium chloride and partly by exchange with intracellular K^+ . The results of a variety of investigations on the distribution of electrolytes, and of water, in the nervous system have been summarized by Van Harreveld (1966) and in brain slices, after incubation in various media, by McIlwain (1963). Some of these results, obtained in investigations with permeability markers such as sucrose, inulin, thiocyanate and chloride, that bear on the studies recorded below,

are as follows. (1) The distribution of Na^+ in the brain tissue is similar to that of Cl^- , but some of the Na^+ may be bound (Van Harreveld, 1966) and some Na^+ may exchange with K^+ (Bachelard, Campbell & McIlwain, 1962). The electrolyte distribution, which is responsible for water movements, is presumably channelled through ion pumps (Pappius, Rosenfeld, Johnson & Elliott, 1958; Bachelard *et al.* 1962; Whittam, 1962) in which ATPase* may be involved (Deul & McIlwain, 1961). Calculations (Keesey & Wallgren, 1965) based on electrochemical gradients suggest that about one-quarter of the energy available from respiration is required for optimum Na^+ and K^+ transport in brain-cortex slices. (2) The uptake of water is associated with the presence of a diffusible external anion (e.g. Cl^-) and may be prevented by the presence of a relatively non-diffusible anion (e.g. isethionate). A linear relationship exists between the size of the chloride space and the amount of water uptake (Bourke & Tower, 1966a). A part of the water uptake is associated with the presence of K^+ in the incubation medium (Pappius

* Abbreviation: ATPase, adenosine triphosphatase.

& Elliott, 1956; Bourke & Tower, 1966a). (3) The water uptake associated with damaged surfaces of the brain tissue is usually small [amounting to less than one-fifth of the inulin space (Varon & McIlwain, 1961; Keesey, Wallgren & McIlwain, 1965; Bourke & Tower, 1966a)]. The inulin (mainly extracellular) space does not vary in the same manner as the extent of water uptake with changed incubation or metabolic conditions (Bourke & Tower, 1966a; Bradford & McIlwain, 1966). (4) The considerable water uptake by brain slices under anaerobic conditions is due to the movement of electrolytes into the tissue and the inability of the cells to extrude Na^+ (Pappius, 1964).

We have carried out investigations to throw further light on the relation of water uptake to the processes involving energy metabolism in the brain. This paper is concerned with measurements of the water uptakes of rat brain-cortex slices incubated under various conditions and the ATP concentrations found in the tissues under these conditions. An attempt is made to explain the apparent relationship existing between the uptakes of water and the ATP concentrations.

MATERIALS

Animals. Male adult rats of the Wistar strain weighing 150–200 g were used. Infant rats (1–3 days old) of the Wistar strain, both male and female, were obtained from the Vivarium, Department of Zoology, University of British Columbia, Vancouver, B.C., Canada.

Radioactive materials. Tritiated water used was an aqueous solution of specific radioactivity 1 mCi/g obtained from New England Nuclear Corp., Boston, Mass., U.S.A. It was diluted with water and used in appropriate quantities.

$^{22}\text{NaCl}$ of specific radioactivity 2.3 mCi/ μmol was obtained in aqueous solution (0.1 mCi/ml) from The Radiochemical Centre, Amersham, Bucks., U.K.

Enzymes. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) type X (from torula yeast) and hexokinase (EC 2.7.1.1) type C-300 (from yeast) were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A.

METHODS

Preparation of brain-cortex slices. Rats were killed by stunning, the brains removed and cerebral-cortex slices prepared with a Stadie-Riggs tissue slicer with a dry razor. Care was taken that the slices were not more than 0.4 mm thick. One dorsal and one lateral slice ('first slices') weighing a total of 60–70 mg wet wt. from the same brain were used.

Infant rats were decapitated, their brains removed and slices of the brain cortex were prepared by cutting by hand, only slices of the temporal-parietal portion of each hemisphere being used. As the infant rat brain is small it was found expedient to use slices up to 1 mm thick, but thinner slices, for direct comparison with adult rat brain-cortex slices, were also used. The wet weights of the slices from each animal ranged from 40 to 60 mg.

Incubation procedure. The slices were weighed at once on a torsion balance and suspended in a Krebs–Ringer phosphate medium of the following composition: 128 mm- NaCl , 5 mm- KCl , 2.8 mm- CaCl_2 , 1.3 mm- MgSO_4 , 10 mm- Na_2HPO_4 (adjusted to pH 7.4 with HCl). Except where otherwise stated, 10 mm-glucose was added to the medium. Various substances were added to this incubation medium, the final volume being 3 ml. The incubation was carried out with the conventional Warburg manometric apparatus in an atmosphere of O_2 at 37°C (except where otherwise stated) for, usually, 60 min.

In anaerobic experiments, a Krebs–Ringer bicarbonate medium of the following composition was used: 122 mm- NaCl , 5 mm- KCl , 1.3 mm- CaCl_2 , 1.3 mm- MgSO_4 , 25 mm- NaHCO_3 , 0.4 mm- Na_2HPO_4 (adjusted to pH 7.4 with HCl) and 10 mm-glucose. The incubation was carried out in an atmosphere of $\text{N}_2 + \text{CO}_2$ (95:5).

Solutions (0.1 ml or 0.2 ml) of radioactive substances were placed in the side tubes of the manometric vessels and added to the medium in the main compartment 5 min after incubation at 37°C had commenced. With tritiated water 5 μCi of $^3\text{H}_2\text{O}$ was added to each manometric vessel; with $^{22}\text{NaCl}$ 1.0 μCi of $^{22}\text{NaCl}$ was added to each vessel. CO_2 , in aerobic experiments, was absorbed by 0.2 ml of 20% (w/v) KOH on small rolls of filter paper placed in the centre wells. Measurement of the rates of oxygen uptake at 37°C were made.

Preparation of different incubation media. (a) K^+ -free media. KCl was omitted from the incubation medium.

(b) Na^+ -free media. NaCl was replaced by LiCl , or by choline chloride, or by tris (adjusted to pH 7.4 with HCl). Sodium phosphate buffer was also replaced by 20 mm-tris buffer (adjusted to pH 7.4 with HCl). For low- Na^+ media only NaCl was replaced and the sodium phosphate buffer was not replaced by the tris buffer.

(c) Low-chloride media. In preparing low-chloride high-sulphate media, NaCl was replaced by Na_2SO_4 and KCl was replaced by K_2SO_4 .

Na_2HPO_4 was replaced by tris buffer (adjusted to pH 7.4 with H_2SO_4) and NaCl was replaced by Li_2SO_4 , or by tris buffer, in low-chloride media devoid of Na^+ .

(d) High- K^+ media. KCl was added to the incubation medium to the desired concentration, without any changes in the other ions present.

(e) Media containing Cu^{2+} , Pb^{2+} or Hg^{2+} ions. Solutions of CuCl_2 , $\text{Pb}(\text{NO}_3)_2$ or HgCl_2 were mixed with solutions of glycylglycine (adjusted to pH 7.4 with HCl), equimolar for Cu^{2+} and Hg^{2+} and 6 mm for Pb^{2+} to prevent precipitation of the metals. They were added to the incubation medium at the commencement of the experiments.

Electrical stimulation. Electrical stimulation of slices was carried out in silver-grid vessels similar to those used by Wallgren & Kulonen (1960) by using electrical impulses from the alternating-frequency generator of Tetratron type (Tetratron Co., Helsingfors, Finland), described in detail by Wallgren (1963). The impulses had a pulse frequency of 100 Hz with 0.5 ms duration, the peak potentials being 4 V. The impulses were displayed on a type 561 oscilloscope during the experiment.

Determination of water uptake. Immediately after the incubation the slices were removed from the media and drained. They were spread without folding on an ice-cold clean glass surface and excess of fluid around the slices and on the glass was absorbed with strips of filter paper.

The slices were further drained by touching the glass surface several times until no cloud formed on the surface. The slices were then weighed on a torsion balance. The difference between this final wet weight and the initial wet weight of the slices gives a measure of the increase of water uptake. This difference multiplied by $100/A$ where A is the initial wet weight of tissue is the amount of water taken up by the tissue per 100mg wet wt.

The total water content of the tissue is equal to the sum of the water taken up by the tissue during the period of incubation and the amount of water originally present, i.e. 80% initial wet weight with adult rat brain or 88.4% initial wet weight with infant rat brain.

Determination of radioactivities of tissues. After the final weights had been determined, the slices were homogenized in 3ml of 5% (w/v) trichloroacetic acid with a Teflon homogenizer and left at room temperature either for 30min when radioactivity due to $^3\text{H}_2\text{O}$ was being assessed or for 2h when that due to $^{22}\text{NaCl}$ was being measured. The homogenates were centrifuged and their radioactivities were measured by using 0.5ml samples of the supernatants. When the radioactivities of the incubation media were determined, 0.1ml or 0.2ml samples were used.

When $^3\text{H}_2\text{O}$ was used the weighed slices were not rinsed before homogenization to prevent loss of $^3\text{H}_2\text{O}$ from the slices. Control experiments showed that such rinsing results in a serious loss of radioactivity from the tissue whereas lack of rinsing introduces a small error, amounting to less than 6% of the radioactivity of the tissue.

In experiments with $^{22}\text{NaCl}$ the final concentration of radioactivity in the incubation medium was $0.33\mu\text{Ci/ml}$. After the tissue slices had been drained and weighed at the termination of the experiment they were briefly rinsed once with chilled phosphate-glucose medium and homogenized in 3ml of 5% (w/v) trichloroacetic acid.

Samples of the supernatant were added to 10ml of scintillant consisting of equal volumes of dioxan, toluene and 95% (v/v) ethanol containing 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.005%), 2,5-diphenyloxazole (0.5%) and naphthalene (8.0%).

The radioactivities were counted in a Nuclear-Chicago Scintillation Counter Mark I. The counting efficiency was corrected for each sample by means of the channels-ratio technique. The usual counting efficiencies were between 9 and 12% for ^3H , and between 21 and 23% for ^{22}Na . The total volume in 100mg initial wet wt. in tissue occupied by $^3\text{H}_2\text{O}$ is given by the expression:

$$\frac{\text{d.p.m./100mg initial wet wt.}}{\text{d.p.m./ml of medium}}$$

The amount of ^{22}Na taken up, in terms of $\mu\text{equiv./g}$ initial wet wt. of tissue, is given by the expression:

$$\frac{(\text{d.p.m./g initial wet wt.}) \times 148}{\text{d.p.m./ml of medium}}$$

as the incubation medium contained $148\mu\text{equiv. of Na}^+/\text{ml}$.

Determination of ATP. ATP was determined in samples of the supernatant by an enzymic method with hexokinase and glucose 6-phosphate dehydrogenase (Greengard, 1963). The tissue slices, at the termination of the experiments, were extracted with 2ml of 6% (w/v) HClO_4 and centrifuged, and the supernatant was neutral-

ized with K_2CO_3 . A 0.2ml sample of the supernatant was used for the ATP assay. The reaction mixture for the enzymic assay consisted of the following: glucose, $215\mu\text{mol}$; NADP^+ , 9.4nmol ; MgCl_2 , $3\mu\text{mol}$; EDTA (pH 7.4), $1.2\mu\text{mol}$; triethanolamine buffer (pH 8.0), $24\mu\text{mol}$; glucose 6-phosphate dehydrogenase, 1.6 unit; hexokinase, 1 unit. The total volume was 2.0ml. Incubation was carried out at room temperature for 30min. NADPH was measured fluorimetrically (Itoh & Quastel, 1970).

ATP concentration was calculated as that amount contained in 100mg initial wet wt. of brain tissue.

Numbers of experiments. All experiments, whose results are given below, were carried out at least three times. The means of these results $\pm$ s.d. are recorded.

RESULTS

Movements of tritiated water ($^3\text{H}_2\text{O}$) into brain slices

Tritiated water has been extensively used for the determination of total body water (Feinendegen, 1967), and Bering (1952), in studies of water exchange between the central nervous system and the cerebrospinal fluid, has shown that deuterated water, after intravenous injection into dogs, rapidly makes its appearance in the brain, the time for cerebral grey matter to reach half the serum concentration of D_2O being 12s.

When slices are incubated in a saline medium containing $^3\text{H}_2\text{O}$, there occurs a rapid exchange between H_2O and $^3\text{H}_2\text{O}$, and at 37°C equilibrium is achieved in less than 2min. Results given in Table 1 show that at 30°C , after incubation for 2min in a physiological saline medium containing 10mm-glucose, the concentration of $^3\text{H}_2\text{O}$ within the slice is 94% of that in the medium. Similar rapid equilibration occurs in the presence of 200mm-sucrose, which brings about shrinking of the tissue, or in the presence of 0.1mm-2,4-dinitrophenol, which promotes swelling (Table 1). When incubation is carried out at low temperature (4°C) the rate of equilibration is diminished, the tissue concentration of $^3\text{H}_2\text{O}$, after 2min, usually being less than 60% of its concentration in the medium. Incubation for 15min is usually sufficient for complete, or almost complete, equilibration at 4°C (Table 1). It is evident that conditions that bring about increased water uptake in incubated slices, such as the presence of 0.1mm-2,4-dinitrophenol or of 0.1mm-ouabain, do not affect the rate of exchange between H_2O and $^3\text{H}_2\text{O}$ (Table 1). It seems, however, that a high concentration of K^+ may diminish the initial rate of equilibration with $^3\text{H}_2\text{O}$ (Table 1) and the reason for this is not clear. The temperature coefficient (Q_{10}) for the process of equilibration between 37 and 4°C is found to be 1.26.

Similar studies carried out with infant rat (3 days

Table 1. *Movements of water and tritiated water into rat brain-cortex slices incubated at 4 and 30°C*

Rat brain-cortex slices were placed in 2.9 ml of Krebs-Ringer phosphate medium containing 10 mM-glucose, to which other substances noted below were added, in the main compartments of Warburg manometric vessels. A 0.1 ml portion of the same medium containing 5 μ Ci of tritiated water ($^3\text{H}_2\text{O}$) was placed in the side tubes of the vessels. After incubation for 5 min after the vessels had been gassed with O_2 for 5 min, the contents of the side tubes were tipped into the main vessels, and incubation at 4°C or 30°C was carried out for 2 min or 15 min. Contents of H_2O and $^3\text{H}_2\text{O}$ were measured as described in the text. The fifth column gives ratios of content of $^3\text{H}_2\text{O}$ in the tissue to that in the incubation medium.

Substance added to the medium	Time of incubation (min)	Total water content ($\mu\text{l}/100\text{mg}$ initial wet wt.)		$^3\text{H}_2\text{O}$ content ($\mu\text{l}/100\text{mg}$ initial wet wt.)		$\frac{[^3\text{H}_2\text{O}] \text{ in tissue}}{[^3\text{H}_2\text{O}] \text{ in medium}}$	
		30°C	4°C	30°C	4°C	30°C	4°C
None	2	88.4 $\pm$ 0.5	89.6 $\pm$ 0.8	82.5 $\pm$ 3.1	49.4 $\pm$ 3.7	0.94 $\pm$ 0.04	0.55 $\pm$ 0.04
	15	93.7 $\pm$ 0.8	92.3 $\pm$ 1.4	94.0 $\pm$ 7.2	83.2 $\pm$ 1.1	1.01 $\pm$ 0.06	0.91 $\pm$ 0.01
100 mM-KCl	2	91.3 $\pm$ 1.6	84.4 $\pm$ 2.4	71.2 $\pm$ 1.2	41.5 $\pm$ 7.0	0.80 $\pm$ 0.03	0.49 $\pm$ 0.07
	15	95.3 $\pm$ 1.9	87.0 $\pm$ 2.4	86.9 $\pm$ 4.8	86.8 $\pm$ 1.9	0.95 $\pm$ 0.10	1.00 $\pm$ 0.05
200 mM-Sucrose	2	71.3 $\pm$ 2.2	70.1 $\pm$ 0.7	68.6 $\pm$ 3.7	51.5 $\pm$ 3.2	0.96 $\pm$ 0.02	0.73 $\pm$ 0.05
	15	72.6 $\pm$ 1.3	70.0 $\pm$ 1.8	68.9 $\pm$ 0.3	73.9 $\pm$ 3.7	0.95 $\pm$ 0.02	1.07 $\pm$ 0.03
0.1 mM-2,4-Dinitrophenol	2	94.7 $\pm$ 1.1	85.8 $\pm$ 0.3	85.4 $\pm$ 3.1	47.6 $\pm$ 0.4	0.90 $\pm$ 0.02	0.56 $\pm$ 0.00
	15	103.8 $\pm$ 1.8	95.4 $\pm$ 1.5	106.6 $\pm$ 4.1	93.0 $\pm$ 1.5	1.03 $\pm$ 0.06	0.98 $\pm$ 0.03
0.1 mM-Ouabain	2	94.8 $\pm$ 4.4	89.7 $\pm$ 4.2	82.2 $\pm$ 12.7	51.0 $\pm$ 8.3	0.87 $\pm$ 0.10	0.57 $\pm$ 0.07
	15	101.6 $\pm$ 3.5	90.5 $\pm$ 0.9	105.6 $\pm$ 3.9	90.9 $\pm$ 4.8	1.04 $\pm$ 0.03	1.00 $\pm$ 0.04

Table 2. *Effects of addition of various oxidizable substrates on the respiration, water uptake and ATP concentrations of rat brain-cortex slices*

Incubation was carried out in O_2 for 1 h at 37°C in Krebs-Ringer phosphate medium as described in the text. Acid substrates were present as the neutral sodium salts.

Substances added to the incubation medium	O_2 uptake ($\mu\text{mol}/\text{h}$ per 100 mg initial wet wt.)	Water uptake ($\mu\text{l}/100\text{mg}$ initial wet wt.)	ATP content ($\mu\text{mol}/\text{g}$ initial wet wt.)
Adult rat			
None	6.0 $\pm$ 0.3	53.3 $\pm$ 2.5	0.59 $\pm$ 0.04
0.2 mM-Glucose	7.5 $\pm$ 0.3	44.0 $\pm$ 6.0	0.73 $\pm$ 0.02
0.5 mM-Glucose	7.7 $\pm$ 0.3	39.1 $\pm$ 3.3	1.10 $\pm$ 0.01
0.8 mM-Glucose	9.3 $\pm$ 0.3	31.8 $\pm$ 3.3	1.37 $\pm$ 0.01
2.0 mM-Glucose	10.1 $\pm$ 0.3	23.8 $\pm$ 4.2	1.45 $\pm$ 0.06
3.0 mM-Glucose	10.7 $\pm$ 0.2	18.7 $\pm$ 2.5	1.59 $\pm$ 0.06
10.0 mM-Glucose	10.4 $\pm$ 0.2	18.8 $\pm$ 0.9	1.66 $\pm$ 0.03
10 mM-Glucose + 0.5 mM-L-glutamate	9.5 $\pm$ 1.1	29.0 $\pm$ 2.3	1.66 $\pm$ 0.06
10 mM-Glucose + 2 mM-L-glutamate	11.2 $\pm$ 0.2	34.0 $\pm$ 5.0	1.47 $\pm$ 0.07
10 mM-Glucose + 5 mM-L-glutamate	9.4 $\pm$ 1.8	42.6 $\pm$ 2.0	1.32 $\pm$ 0.04
10 mM-Glucose + 10 mM-L-glutamate	10.9 $\pm$ 0.5	48.6 $\pm$ 1.3	1.05 $\pm$ 0.11
10 mM-L-Glutamate	9.3 $\pm$ 0.3	47.6 $\pm$ 3.5	0.74 $\pm$ 0.02
10 mM-Succinate	6.3 $\pm$ 0.4	47.8 $\pm$ 3.9	0.75 $\pm$ 0.03
10 mM-Pyruvate	11.2 $\pm$ 1.0	30.0 $\pm$ 2.5	1.18 $\pm$ 0.04
Infant rat			
None	3.1 $\pm$ 0.5	7.3 $\pm$ 2.6	0.92 $\pm$ 0.02
10 mM-Glucose	3.8 $\pm$ 0.2	0.0 $\pm$ 6.2	2.49 $\pm$ 0.04

old) brain-cortex slices show that the rate of exchange of $^3\text{H}_2\text{O}$ with H_2O is appreciably lower than with adult rat brain-cortex slices. This amounts, at 37°C and with slices of 0.4 mm thickness, to 28% of that found with adult brain. Moreover

the temperature coefficient (Q_{10}) for the process of equilibration in incubated infant rat brain slices between 37 and 4°C is 1.76. The results suggest that differences between the structures of brain cell membranes of immature and adult rats cause

Fig. 1. Relations between ATP concentration and water uptake in incubated rat brain-cortex slices. Line A, in the presence of various concentrations of glucose (○) as shown in Table 2; line B, in the presence of 10mM-glucose and various concentrations of L-glutamate (●) as shown in Table 2; line C, in the presence of 10mM-glucose and various concentrations of 2,4-dinitrophenol (▲) as shown in Table 4.

differences between the rates of penetration of water through the membrane. As with adult rat brain slices, the rates of exchange between $^3\text{H}_2\text{O}$ and H_2O in infant rat brain slices are unaffected by the presence of 0.1mM-ouabain or 0.1mM-2,4-dinitrophenol.

Correlation between water uptake and ATP concentrations in brain-cortex slices under various incubation conditions

Effects of different substrates and substrate concentrations. Results showing the variations of ATP concentrations in adult rat brain-cortex slices incubated in the presence of various concentrations of glucose and of mixtures of glucose and L-glutamate are given in Table 2. Table 2 also shows the variations of the amounts of water uptake under these conditions and the changes in respiratory rates. Plots are shown in Fig. 1 of the water uptakes and the corresponding ATP concentrations under the conditions given in Table 2. An approximately linear relationship exists between the decrease in water uptake and the increase in ATP concentration as the concentration of glucose in the incubation medium increases. A parallel linear relationship also exists between the increase in water uptake and the fall in ATP concentrations in

the presence of 10mM-glucose and of increasing quantities of L-glutamate (Fig. 1).

Statistical analyses of the results (Table 2) obtained with different concentrations of glucose, or with different concentrations of L-glutamate in the presence of a constant concentration (10mM) of glucose, indicate a linear relationship in each condition between water uptake (Y) and ATP concentration (X). These are: line A: $Y = -32.3X + 72.4$; coefficient of correlation -0.975 ; line B: $Y = -30.6X + 80.6$; coefficient of correlation -0.907 . The difference between the coefficients of X is not significant ($P > 0.25$), indicating that the two lines are approximately parallel. The lines are, however, significantly displaced one from another ($P < 0.005$).

These values apply to results obtained after incubation for 1h. Incubation for a long period results in further increases of water uptake (Pappius & Elliott, 1956). We have not, however, made measurements after 1h incubation because prolonged incubation tends to bring about disintegration of the slices.

Experiments with brain-cortex slices from infant rats show (Table 2) that only little water uptake occurs either with or without glucose (see also Tower & Bourke, 1966), and this may be correlated with the fact that the water content of infant rat brain is normally considerably higher than that of adult rat brain. The results show that the amount of water present in slices from infant rat incubated for 1h in the absence of glucose is 89% of the final wet weight. This value approximates closely to that found in slices from adult rat (87%) incubated under similar conditions.

Effects of substitution of glucose by sodium pyruvate, L-glutamate or succinate. Results given in Table 2 show that sodium pyruvate (10mM) is not as effective as glucose (10mM) either in suppressing the water uptake found on incubation in a medium devoid of oxidizable substrates or in increasing the concentration of brain-cell ATP. It is, however, much more effective, in these respects, than L-glutamate or succinate. Neither of these substances affects appreciably either the water uptake or the ATP concentration found in the absence of glucose.

Time-course of uptakes of water and of $^{22}\text{Na}^+$ into slices incubated under anaerobic conditions. It is well known that under anaerobic conditions there is extensive uptake of water into incubated brain slices with influx of Na^+ (Van Harreveld, 1966; Bourke & Tower, 1966). Results indicating this fact are recorded in Table 3.

It will be observed that the quantity of $^{22}\text{Na}^+$, expressed as $\mu\text{equiv./ml}$ of tissue fluid, in the total water present in the brain tissue, assuming that the initial quantity of water in the slices is 80% of the wet weight, becomes constant after the first 15 min of anaerobic incubation. Moreover this amount is

Table 3. *Time-course of uptake of water and of $^{22}\text{Na}^+$ into rat brain-cortex slices incubated under anaerobic conditions*

Incubation was carried out in a Krebs–Ringer bicarbonate medium containing 10mM-glucose in an atmosphere of $\text{N}_2 + \text{CO}_2$ (95:5) at 37°C for 45 min: 1.0 μCi of $^{22}\text{NaCl}$ was present in the side tube of the Warburg manometric vessel and tipped into the main compartment after temperature equilibration. Further details are given in the text.

Time (min)	Water uptake ($\mu\text{l}/100\text{mg}$ initial wet wt.)	$^{22}\text{Na}^+$ taken up ($\mu\text{equiv.}/\text{g}$ initial wet wt.)	Total water present in tissue ($\mu\text{l}/\text{g}$ initial wet wt.)	$^{22}\text{Na}^+$ taken up ($\mu\text{equiv.}/\text{ml}$ of tissue fluid)
3	20.3	99.6	1003	99.3
6	23.9	129.8	1039	124.9
15	32.5	174.3	1125	154.9
30	37.0	184.6	1170	157.8
45	47.9	198.3	1279	155.0

Table 4. *Effects of metabolic inhibitors on the water uptake and ATP concentrations of incubated rat brain-cortex slices*

Incubation was carried out in presence of O_2 at 37°C for 1 h in a Krebs–Ringer phosphate medium containing 10mM-glucose. Copper, mercury or lead salts were added, in the presence of glycylglycine, pH 7.4, as described in the text.

Additions to the incubation medium together with 10mM-glucose	O_2 uptake ($\mu\text{mol}/\text{h}$ per 100mg initial wet wt.)	Water uptake ($\mu\text{l}/100\text{mg}$ initial wet wt.)	ATP content ($\mu\text{mol}/\text{g}$ initial wet wt.)
None	10.4 $\pm$ 0.1	18.8 $\pm$ 0.9	1.66 $\pm$ 0.03
0.01 mM-2,4-Dinitrophenol	14.1 $\pm$ 0.4	26.0 $\pm$ 2.8	1.26 $\pm$ 0.08
0.02 mM-2,4-Dinitrophenol	16.4 $\pm$ 0.8	28.3 $\pm$ 1.5	1.09 $\pm$ 0.06
0.03 mM-2,4-Dinitrophenol	16.9 $\pm$ 1.6	29.9 $\pm$ 3.3	1.08 $\pm$ 0.03
0.05 mM-2,4-Dinitrophenol	12.6 $\pm$ 0.6	34.6 $\pm$ 3.3	0.79 $\pm$ 0.01
0.07 mM-2,4-Dinitrophenol	12.7 $\pm$ 0.3	39.2 $\pm$ 2.8	0.77 $\pm$ 0.02
0.10 mM-2,4-Dinitrophenol	8.8 $\pm$ 0.4	44.5 $\pm$ 2.0	0.62 $\pm$ 0.02
0.1 mM-Ouabain	10.5 $\pm$ 0.5	43.4 $\pm$ 2.1	1.35 $\pm$ 0.04
0.3 mM- CuCl_2	9.6 $\pm$ 0.4	39.7 $\pm$ 0.9	1.20 $\pm$ 0.25
0.3 mM- HgCl_2	8.1 $\pm$ 0.1	40.2 $\pm$ 1.0	1.01 $\pm$ 0.02
0.3 mM- PbCl_2	10.1 $\pm$ 0.2	18.0 $\pm$ 4.7	1.71 $\pm$ 0.10
2 mM-Sodium malonate	7.1 $\pm$ 0.2	29.1 $\pm$ 0.4	1.13 $\pm$ 0.04
10 mM-Sodium D-glutamate	8.3 $\pm$ 0.1	44.8 $\pm$ 3.9	0.75 $\pm$ 0.02

approximately equal to the concentration of $^{22}\text{Na}^+$ in the incubation medium.

Effects of metabolic inhibitors

2,4-Dinitrophenol. The effects of the presence of various concentrations of 2,4-dinitrophenol on the respiration, water uptake and the ATP content of slices incubated in a medium containing glucose are shown in Table 4. As the ATP concentration gradually diminishes, with increasing concentration of 2,4-dinitrophenol, the extent of water uptake gradually increases. The relation between ATP concentration and water uptake is plotted in Fig. 1, where it is evident that an approximately linear relationship exists. The plot (line *C*, Fig. 1) may be expressed by the equation $Y = -28.6X + 60.8$

(coefficient of correlation -0.903). The value of the coefficient of X for line *C* is not significantly different from that of X in either line *A* or line *B* ($P > 0.25$).

Ouabain. The presence of 0.1mM-ouabain exerts a marked increase on the extent of water uptake concomitant with only a relatively small decrease in the ATP concentration (Table 4). The rate of oxygen consumption is unaffected under the conditions of these experiments. Bourke & Tower (1966b) have demonstrated that ouabain, at concentrations exceeding 0.01mM, greatly increases the water uptake of incubated cortex slices from cat brain and that the increased water uptake takes place in the intracellular spaces of the brain tissue.

With brain slices from infant rats, incubated in a 10mM-glucose medium, the presence of ouabain

(0.1mm) increases the water uptake by 14% with but little effect on the rate of respiration.

Cu²⁺, Hg²⁺ and Pb²⁺ ions. Both Cu²⁺ and Hg²⁺ bring about (at a concentration of 0.3mm) very marked increases in the water uptake (Table 4). There occur also decreases in the concentrations of ATP, but the decreases are not as great as those taking place in the presence of those concentrations of 2,4-dinitrophenol that bring about equal increases in the water uptake. Only relatively small decreases in the rates of oxygen consumption take place. The effects of Cu²⁺ and Hg²⁺ resemble those of ouabain. Pb²⁺ (0.3mm) exerts no effects, under the given experimental conditions, on the rate of oxygen consumption, on the water uptake or on the ATP concentration (Table 4). Cu²⁺ (1mm) markedly increases the water uptake of infant rat brain-cortex slices incubated in a glucose (10mm) medium. The increase amounts to 17.6% and there is no decrease in the rate of respiration.

D-Glutamate. The effects of D-glutamate are included in Table 4 as this substance affects L-glutamate metabolism in the brain without itself undergoing marked metabolic changes. The results show that the presence of D-glutamate brings about

a considerable increase in the water uptake of rat brain-cortex slices incubated in a glucose-saline medium with a concomitant large decrease in the brain ATP concentration. There is a relatively small decrease in the rate of oxygen consumption (Table 4).

Sodium malonate. The presence of sodium malonate (2mm), which brings about a decrease in the rate of oxygen consumption, causes an increased uptake of water to take place with a decrease in the ATP concentration (Table 4).

Effects of various concentrations of cations and anions

Effects of changes of K⁺ concentration. The well-known dependence of water uptake on the K⁺ content of the incubation medium is shown in the results given in Table 5. Also, either the absence of K⁺ or the presence of a high concentration of K⁺ leads to a marked increase of water uptake with an accompanying decrease in the ATP concentration.

Effects of replacement of Na⁺ by Li⁺, choline or tris ions. Replacement of Na⁺ by Li⁺ causes

Table 5. *Effects of changes in concentrations of anions and cations in the incubation medium on the rate of respiration, water uptake and ATP concentrations in rat brain-cortex slices*

Incubation was carried out in a saline-10mm-glucose medium at 37°C in O₂ for 1 h. All media contained 1.3mm-MgSO₄, 2.8mm-CaCl₂ and 10mm-sodium phosphate buffer, pH 7.4. All cations were present as their chlorides, except as noted in the table. Media containing tris buffer have no phosphate present.

Concentrations in incubation medium (mm)					O ₂ uptake (μmol/h per 100 mg initial wet wt.)	Water uptake (μl/100 mg initial wet wt.)	ATP content (μmol/g initial wet wt.)
Cations							
Na ⁺	K ⁺	Li ⁺	Choline ⁺	Tris ⁺			
148	5	—	—	—	10.4±0.1	18.8±0.9	1.63±0.03
148	—	—	—	—	10.9±0.2	30.4±0.7	0.91±0.09
128	5	—	—	20	9.0±0.6	20.0±0.7	1.50±0.06
25	5	—	113	—	11.0±0.3	18.7±0.3	1.70±0.10
—	5	128	—	20	10.5±0.3	46.6±2.3	0.78±0.05
—	5	—	128	20	7.9±0.1	32.8±1.3	0.87±0.06
—	5	—	—	148	7.9±0.1	50.3±3.3	0.71±0.03
148	105	—	—	—	14.0±0.1	40.4±1.2	1.20±0.05
Anions (in the presence of the normal cation concentrations)							
Cl ⁻		SO ₄ ²⁻					
148		1.3			10.4±0.1	18.8±0.9	1.63±0.03
5.6		77.8			8.5±0.9	9.4±0.8	1.06±0.04
5.6		77.8		20	7.4±0.3	7.4±0.6	0.51±0.04
(Na ⁺ replaced by 128mm-Li ⁺)							
5.6		77.8		148 (no Na ⁺ present)	6.8±0.1	8.4±0.3	0.59±0.09

Table 6. *Effects of electrical stimulation on the rate of respiration, water uptake and ATP concentration of rat brain-cortex slices*

Incubation was carried out in Warburg manometric vessels, equipped with silver-wire electrodes, in Krebs-Ringer phosphate medium containing 10mm-glucose at 37°C in O₂ for 1 h. For further details see the text.

Electric pulses	O ₂ uptake (μ mol/h per 100mg initial wet wt.)	Water uptake (μ l/100mg initial wet wt.)	ATP content (μ mol/g initial wet wt.)
None	9.2 $\pm$ 0.6	19.2 $\pm$ 2.3	1.57 $\pm$ 0.03
4 V	12.9 $\pm$ 0.4	31.7 $\pm$ 2.4	1.05 $\pm$ 0.06

increased uptake of water [as also noted by Bourke & Tower (1966a) for incubated brain-cortex slices from the cat] together with a decrease in the ATP concentration. Replacement of Na⁺ by choline ions also leads to increased water uptake and diminished ATP concentration (see also Bourke & Tower, 1966a), but the presence of choline has no effect on the water uptake when 25mequiv. of Na⁺/l are present (Table 5).

Replacement of Na⁺ by tris ions leads to almost maximum values of increased water uptake and to diminished ATP concentrations.

The presence of SO₄²⁻ as the main anion of the incubation medium suppresses water uptake, even under conditions where all the Na⁺ in the medium is replaced by Li⁺ or by tris (Table 5). The effect resembles that due to isethionate with incubated cat brain-cortex slices (Bourke & Tower, 1966b). Nevertheless, the substitution of SO₄²⁻ for most of the Cl⁻ in the incubation medium does not prevent the decrease in ATP concentration that takes place when Na⁺ in the medium is replaced by Li⁺ or by tris (Table 5). Such results may be explained on the view that although SO₄²⁻, as a relatively non-diffusible anion, prevents the influx of cations into the tissue, it does not prevent the efflux of cations from the tissue, with resultant adverse effects on the rates of respiration and on the ATP concentrations.

Effects of electrical impulses

Application of electrical impulses for 1 h to rat brain-cortex slices incubated in a Krebs-Ringer phosphate medium containing 10mm-glucose results in increased water uptakes and diminished ATP concentration (Table 6).

DISCUSSION

Water exchange and metabolic inhibitors. The flow of water through brain-cell membranes, as indicated by the exchange of ³H₂O with H₂O in rat brain-cortex slices incubated in a medium containing

glucose, takes place rapidly and seems to be unaffected by the presence of metabolic inhibitors (ouabain, 2,4-dinitrophenol). It is therefore evident that conditions greatly affecting water uptake do not necessarily affect the interflow of water across the cell membrane. The composition of the membrane structure, however, affects the exchange process.

Effects of glucose, L-glutamate and other substrates. Incubation of brain slices in media containing different concentrations of glucose shows no change either in the amount of water taken up, or in the brain ATP concentrations, until the glucose concentration falls below 3mm. At about this concentration of glucose Joanny & Hillman (1964) observed almost the maximum respiratory rate and the maximum K⁺ and Na⁺ gradients between slice and medium with guinea-pig brain. Below this concentration of glucose the amount of water uptake by rat brain-cortex slices gradually increases and the ATP concentration decreases until the maximum values formed in the complete absence of glucose are reached. The relationship between increase of water uptake and decrease of ATP concentration is approximately linear (Fig. 1). If glucose is replaced by pyruvate, L-glutamate or succinate, there are increased values of water uptake and diminished values of ATP concentration, and the relationship between these values seems to be similar to that found with different concentrations of glucose (Fig. 1).

When L-glutamate is added at different concentrations, in the presence of 10mm-glucose, there appears also to be an approximately linear relationship between the values of water uptake and ATP concentration, but the line is parallel to that found with changed glucose concentrations and shifted significantly in the direction of increased water uptake for the same ATP concentration (Fig. 1).

Relation between water uptake and ATP concentration. The linear relationship existing between the extent of water uptake and the ATP concentration may be explained by assuming that the amount of water uptake is proportional to the net

influx of cations into the brain tissue and that the ATP concentration and the activity of $\text{Na}^+ + \text{K}^+$ -stimulated ATPase partly control the cationic fluxes. This follows from a consideration of reactions involved in the sodium pump, which, following the suggestions of Skou (1965), Whittam (1967) and Sen, Tobin & Post (1969), may be partly represented as follows:

Such a scheme postulates phosphorylation of a component of $\text{Na}^+ + \text{K}^+$ -stimulated ATPase, namely E, which combines with internal Na^+ (reaction 1) and subsequent breakdown of the combination, after conformational change, to liberate Na^+ which is released into the incubation medium (reaction 2). The scheme also envisages combination of external K^+ with the phosphorylated component of E and, after conformational change, liberation of K^+ into the cell. Assuming that reaction (1) is rate-limiting, it follows that both the rates of efflux of Na^+ and of influx of K^+ will be proportional to the ATP concentration and to the effective concentration of E.

Effects of diminished extrusion of Na^+ . As shown by Leaf (1956) in his analysis of the effects of disturbed metabolism on the ion content of the cell, accumulation of Na^+ has, as a consequence, an increase of tissue Cl^- . The temporary rise in osmotic pressure thus produced manifests itself as an increase of tissue hydration. This may also be seen in the following manner. With a decrease in the active outward transport of Na^+ , the intracellular concentration of Na^+ will rise and, correspondingly, the concentration of K^+ will fall. This, in itself, would cause no net influx of water unless at least some of the cells in the slices are permeable to extracellular anion, e.g. Cl^- . In this case, the decrease of resting potential due to diminished internal K^+ implies influx of Cl^- in accordance with the Donnan equilibrium. This can only occur with a corresponding influx of cation, i.e. Na^+ and, therefore, of water to preserve osmotic equilibrium across the membrane. The net influx of water will therefore parallel the loss of K^+ from the cells. The experimental results showing an inverse linear relation between water uptake and ATP concentration thus support the reaction sequence given above. It is, however, evident from a consideration

of enzyme kinetics that proportionality to ATP concentrations will only occur at concentrations below the saturation value of ATP for membrane ATPase. The concentration of ATP at the cell membrane is not known but perhaps it may be assumed that it is proportional to the brain cell concentration of ATP. An affinity constant, K_m , for the reaction between ATPase and ATP is quoted as 0.4mM (Robinson, 1967). Such a value would indicate that the concentration of ATP at the brain-cell membrane is smaller than the value of 1.7mM found for the cell concentration of ATP. This matter can only be resolved when it is possible to assess the actual ATP concentration at the site of the $\text{Na}^+ + \text{K}^+$ -activated ATPase, presumably at the brain-cell membrane.

Water uptake by infant and adult rat brain. Note may be made of the finding that the amount of water present in infant brain tissue incubated for 1h in the absence (or presence) of glucose is approximately the same as that found in adult brain tissue incubated for 1h in the absence of glucose. This equivalence may be largely due to the relative lack of $\text{Na}^+ + \text{K}^+$ -stimulated ATPase activity in infant rat brain (Samson & Quinn, 1967; Abdel-Latif, Brody & Ramahi, 1967), which doubtless results in effects similar to those brought about in adult rat brain incubated in the absence of glucose.

Effects of induced influx of Na^+ . Induced influx of Na^+ after alteration of membrane permeability may, by stimulation of the activity of the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase, bring about a diminution of the ATP concentration. Under such circumstances it will be expected that there will be a correspondence between the increased electrolyte influx (and therefore the resulting water uptake) and the decrease in ATP concentration. Although the ATP concentration, under these conditions, exerts no control on the initial Na^+ influx, it becomes important in the control of Na^+ efflux. It will therefore have a controlling effect on the final steady state involving electrolyte influx and water uptake.

Adherent water. It should be borne in mind, in considering the apparent linear relationship existing between the extent of water uptake and the ATP concentration, that a portion of the water uptake is due to adherence of water to the cut surfaces, or damaged cells, of the tissue slices. This amount of water is, however, as already mentioned, relatively small, amounting to less than one-fifth of the inulin space, and its uptake takes place rapidly after immersion of the tissue into the incubation medium. We have found, under our experimental conditions, that it does not exceed 5 $\mu\text{l}/100\text{mg}$ wet wt. of tissue. It is unlikely to be affected appreciably by the changes in incubation or metabolic conditions considered here. Therefore correction of the total water uptake by the amount of this 'adherent'

water will not significantly affect the linear relationship that has been described.

Intracellular water. The proportionality that apparently exists between the water uptake and the ATP concentration suggests that much of the water uptake is intracellular. Zadunaisky, Wald & De Robertis (1965) conclude that the gain in water of isolated brain tissue is due to retention of salts largely by glial cells as the neuronal elements are supposed not to be so reactive to water and salt modifications (see also Bakay, 1965; Tower, 1967). The possible role of glial cells in water-ion movements in brain slices is discussed in the following paper (Okamoto & Quastel, 1970).

Effect of anaerobiosis. Results (Table 3) show that, after a period of 15 min anaerobic incubation, the Na^+ concentration in the tissue fluid is approximately the same as that in the incubation medium. Although iso-osmoticity is thus maintained, it is evident that more $^{22}\text{Na}^+$ is taken up than is equivalent to the water uptake. This follows from a simple calculation of the $^{22}\text{Na}^+$ present in the water taken up by the tissue, assuming this to be iso-osmotic with the incubation medium. For example (Table 3), after 30 min anaerobic incubation, $37.0\mu\text{l}$ of water is taken up/100 mg initial wet wt. of tissue; $37.0\mu\text{l}$ of the incubation fluid (148 mM-sodium chloride) contains $5.48\mu\text{equiv.}$ of $^{22}\text{Na}^+$. There is therefore an influx of $54.8\mu\text{equiv.}$ of Na^+/g initial wet wt. of tissue. The actual influx of $^{22}\text{Na}^+$, as seen in the results given in Table 3, corresponding to the uptake of $37.0\mu\text{l}$ of fluid, is $184.6\mu\text{equiv.}$, so that the $^{22}\text{Na}^+$ uptake, corrected for that in the water absorbed, is $184.6 - 54.8 = 129.8\mu\text{equiv.}/\text{g}$. This result may be explained by the fact that the corrected $^{22}\text{Na}^+$ uptake represents the sum of that quantity of $^{22}\text{Na}^+$ exchanging with unlabelled Na^+ together with that replacing K^+ lost during anaerobiosis. A similar conclusion follows from the results of Bourke & Tower (1966a), who used cat brain-cortex slices, where the influx of Na^+ is accompanied by an efflux of K^+ . The net electrolyte influx, which determines the water uptake, is usually less than that indicated by the influx of $^{22}\text{Na}^+$ and is probably better represented by the influx of Cl^- (Bourke & Tower, 1966a).

Effects of L- and D-glutamate. Examination of the effects of different concentrations of L-glutamate, in the presence of 10 mM-glucose, shows (Table 2) that, although proportionality between water uptake and ATP concentration exists, there is in fact a larger water uptake, for a given ATP concentration, than occurs in the absence of added L-glutamate. It is possible to account for this on the basis of the increased Na^+ influx that is known to occur in incubated nervous tissue in the presence of L-glutamate (Ames, 1956; Curtis, Phillips & Watkins, 1960; Krnjevic & Phillips, 1963; Bourke

& Tower, 1966a; Bradford & McIlwain, 1966; Harvey & McIlwain, 1968; Ames, Tsukada & Nesbitt, 1967; Pull, McIlwain & Ramsay, 1970). There is not necessarily an equal K^+ efflux. It seems, in fact, that the initial increase in permeability to Na^+ is not associated with a change in permeability to K^+ (Bradford & McIlwain, 1966).

D-Glutamate, which has initial effects on sodium influx into rabbit retinas similar to those brought about by L-glutamate (Ames, 1956), and which affects glucose metabolism in guinea-pig brain-cortex slices (Takagaki, Hirano & Nagata, 1958), is as effective as L-glutamate in promoting water uptake in rat brain-cortex slices incubated aerobically in a glucose medium (Table 4). It lowers the rate of respiration, and the ATP concentration is diminished to a greater extent than that brought about by an equivalent concentration of L-glutamate. The increased fall in the ATP concentration may perhaps be partly accounted for by the diminished rate of brain respiration.

Effects of 2,4-dinitrophenol. Examination of the results obtained with different concentrations of 2,4-dinitrophenol in the presence of 10 mM-glucose shows a linear relationship between water uptake and ATP concentration similar to that found with different glucose concentrations, but the amount of water uptake is significantly smaller ($P < 0.005$), at a given ATP concentration, in the presence of 2,4-dinitrophenol than that found in its absence. This effect of 2,4-dinitrophenol may be due to the increased efflux of K^+ taking place in its presence (Turner, Eggleston & Krebs, 1950; Whittam & Davies, 1954).

Effects of ATPase inhibitors. Ouabain (0.1 mM) brings about a large increase of water uptake in incubated brain slices (Bourke & Tower, 1966b) with a relatively small decrease in brain ATP concentration (Table 3). This may be explained as due to inhibition of the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase with resultant depression of Na^+ efflux.

Copper chloride (0.3 mM) behaves similarly to ouabain so far as water uptake and ATP concentration in the brain cell are concerned (Table 4) and a similar explanation for its effects may be given. It is known that Cu^{2+} has inhibitory effects on microsomal or membrane ATPase (Peters, Shorthouse & Walshe, 1966; Epstein & McIlwain, 1966), but the effects of Cu^{2+} on ATPase activities are not as specific as those of ouabain (Epstein & McIlwain, 1966).

The fact that both Cu^{2+} and ouabain have enhancing effects on the water uptake of infant rat brain slices indicate that the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase activity of immature brain, though less than that of adult brain, has an important controlling effect on the ionic gradients of infant rat brain.

Effect of mercuric chloride. This substance (0.3mM) seems to be at least as potent as copper chloride in inducing water uptake in incubated adult rat brain slices (Table 4), but we have no information on its effects on Na^+ + K^+ -stimulated ATPase.

Effects of cationic changes in the medium. Omission of K^+ from the medium containing glucose in which the brain slices are incubated results in a lowered ATP concentration and an increased water uptake (Table 5), but the increase in water uptake resembles that taking place in the presence of 2,4-dinitrophenol for the same brain-cell concentration of ATP (Table 4). This may be due to an increased efflux of brain-cell K^+ into the incubation medium, resulting from partial inhibition of the Na^+ + K^+ -stimulated ATPase by diminished extracellular K^+ .

Replacement of Na^+ in the incubation medium by Li^+ or by tris causes electrolyte influx into the brain cell, with no concomitant efflux as these ions are not affected by the sodium pump. There is in consequence increased water uptake (Table 5). A decrease in the brain-cell ATP concentration also takes place. Choline ions do not seem to be as effective as Li^+ in promoting water uptake, and so far we have no explanation for this.

A high external concentration of K^+ (105mequiv./l) promotes an increase of water uptake, with a concomitant decrease in ATP concentration, and the results (Table 5) resemble those due to the addition of L-glutamate (Table 2). It seems possible that its effects are largely due to retention (or promotion) of a high cell K^+ content, together with diminished efflux (or increased influx) of Na^+ , owing to diminished ATPase activity consequent on the decrease of the cell concentration of ATP.

Effects of electrical impulses. The effects of application of electrical impulses (Table 6) may be understood as being due to increases in the influx of Na^+ and in the efflux of K^+ . For the same concentration of ATP in the cell, however, there seems to be a smaller increase in the uptake of water than that brought about by diminution of the glucose concentration in the incubation medium (Fig. 1).

Categories of substances (or conditions) affecting cerebral water uptake. The factors influencing electrolyte influx, and thereby water uptake, into incubated brain slices may be divided into two main categories.

(1) Those substances or conditions that affect permeability of the cell membrane by alteration of its structure so that Na^+ moves into the cell down its electrochemical gradient. With such induced influx of Na^+ the energetics of the cell are not primarily affected. L-Glutamate and the application of electrical impulses belong in this class.

(2) Those substances or conditions that affect the energetics of the cell by their modification of the

activity of the sodium pump, either by effects on Na^+ + K^+ -stimulated ATPase or on the synthesis or breakdown of ATP. Here there is diminished efflux of Na^+ . This class will include substances such as ouabain, Cu^{2+} or 2,4-dinitrophenol or absence from the incubation medium of Na^+ or K^+ .

Factors that result in the retention in the cell of its initial high concentration of K^+ must also be considered. Included in this category are high concentrations of K^+ in the incubation medium.

This classification of factors influencing cerebral water uptake is, however, by no means rigid. This is due to the overlap of their effects, or because of their lack of specificity. Some discrimination between these factors is possible by the use of the neurotropic drug, tetrodotoxin, which exerts specific effects. Results obtained with this substance are described in the following paper (Okamoto & Quastel, 1970).

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