The Sodium and other Ions of Mammalian Cerebral Tissues, Maintained and Electrically Stimulated *in vitro*

By H. S. BACHELARD, W. J. CAMPBELL AND H. McILWAIN

Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, London, S.E. 5

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A previous examination of ion movements in electrically excited cerebral tissues (Cummins & McIlwain, 1961) was primarily concerned with potassium salts and demonstrated their rapid loss from the tissue on excitation, and their rapid return during a subsequent recovery period. Comparable information with respect to the movement of sodium salts has now been sought.

The normal preponderance of sodium salts in fluids bathing the tissue necessitated an initial appraisal of fluid spaces in the tissue, and these were accordingly examined in relation to electrical excitation by Varon & McIlwain (1961). Sodium salts are required in external fluids if the tissue is to regain its resting membrane potential (Hillman & McIlwain, 1961) and to give its normal response to excitation (Gore & McIlwain, 1952), a relationship further examined in the present study. The tissue as ordinarily handled *in vitro* swells and carries markedly more sodium and chloride than does the brain *in vivo* (Leaf, 1956); at the beginning of the present studies, conditions were therefore sought for minimizing such changes.

EXPERIMENTAL

Tissue preparations

Serum. Guinea-pig blood was kept at 37° for 10 min. and then at 3° for a few hours until the clot contracted, yielding serum which was used after it had been adjusted to pH 7.3 and glucose had been added to 10 mM.

Cutting cerebral tissue. Slices (40–60 mg.) of guinea-pig cerebral cortex were prepared for a given experiment in one of the following three ways.

(i) Removed and cut with fluid: the brain was removed and slices were cut with a blade and glass guide lubricated with the incubation medium as described by Cummins & McIlwain (1961). These slices were floated to a dish of the fluid and drained from superficial fluid by repeated contact with a glass surface before weighing.

(ii) Removed and cut fresh: the brain was removed and slices were cut with a bow-cutter and glass guide as described by McIlwain (1961*b*), except that the guide was given a water-repellent surface by treatment with a silicone preparation. These slices were weighed initially without any contact with aqueous fluid; uptake of fluid alters values expressed in terms of tissue weight in fashions detailed by Varon & McIlwain (1961).

(iii) Cut *in situ*: guinea pigs were either stunned and exsanguinated as described by Cummins & McIlwain (1961), or were anaesthetized in a jar containing a pad with CHCl_3 until the eye reflex was barely perceptible. In both cases the skull was opened carefully and immediately on cutting the dura slices were cut from the cortex with a blade and a small guide of the type described by McIlwain (1961*b*), without added fluid. The slices were immediately released to a dish of continuously oxygenated incubating medium at 37°. From there they were picked up in rapid-transfer holders and incubated as described by Varon & McIlwain (1961).

Subsequent treatment of the slices. Slices prepared by methods (i) and (ii) were placed in conical electrode vessels of 15–20 ml. with 3.5 ml. of medium, or in rapid-transfer holders (Cummins & McIlwain, 1961) and incubated at 37° within 20 min. of the animal's death. Slices prepared *in situ* (method iii) were, with the exception of the studies on preparation time (Fig. 2), incubated within 5 min. of exsanguinating or anaesthetizing the animal.

Incubation was in bicarbonate-buffered medium (Varon & McIlwain, 1961) with $\text{O}_2 + \text{CO}_2$ (95:5) as gas phase, or in tris-buffered medium with O_2 as gas phase. The media in some cases contained 1% of inulin; subsequently slices were either drained and reweighed as in (i), or rinsed by release into 0.3M-sucrose and after a few seconds removed for analysis. In some cases the slices cut *in situ* were not weighed at any stage and results are expressed in terms of the protein content of the incubated tissue.

Analytical methods

Slices were ground in 4 ml. of 6% (w/v) trichloroacetic acid, and Na^+ , K^+ and Cl^- ions and inulin were determined as described by Varon & McIlwain (1961); protein was determined in the precipitate by the method of Lowry, Rosebrough, Farr & Randall (1951). Water was determined by the loss of weight at 105° after 90 min. For total lipid, portions of tissue of about 0.5 g. were ground in 19 vol. of CHCl_3 -methanol (2:1, v/v), the suspension was passed through a sintered-glass filter and the filter washed with a further 2 vol. of the same solvent. The clear filtrate with washings was shaken with 4 vol. of 0.1M-KCl, and the lower phase was separated, washed twice with 10.6 vol. of CHCl_3 -methanol-0.1M-KCl (3:48:47, by vol.; see Folch, Lees & Sloane-Stanley, 1957) and evaporated in a stream of air. The residue was extracted with 5 vol. of CHCl_3 -methanol (2:1, v/v) and the lipid left on evaporation weighed: six samples of guinea-pig cerebral cortex gave 72 ± 7 mg./g. of fresh tissue.

Blood was sampled on exsanguination, or from the heart if animals were lightly anaesthetized with CHCl_3 , 5 ml. of

blood then being withdrawn into a syringe containing 50 units of heparin (Liquemin, Roche) in 10 μ l. The mixture was transferred to a centrifuge tube, plasma was separated and Na^+ , K^+ and Cl^- ions were determined by the methods given above; correction was made for the ion content of the heparin.

The fraction IV-4 of blood plasma proteins was obtained from Armour Laboratories and is described by Marks & McIlwain (1959). The specimen of gangliosides yielded sialic acid equivalent to 30.6% of *N*-acetylneuraminic acid and was prepared from ox brain by Mr D. A. Booth (Booth, 1962), whom we thank. Ascorbic acid, adenosine, creatine, guanosine and choline chloride were from Hopkin and Williams Ltd., Chadwell Heath, Essex. Choline bicarbonate was obtained from Ward, Blenkinsop and Co., London, and an aqueous 0.65M solution was saturated with CO_2 immediately before use as a medium constituent.

Calculations

Relationships between different measures of the quantity of tissue. The weight of tissue cut without fluid (method ii) was measured directly whenever possible, as in the experiments of Fig. 1 and Tables 1 and 3, and was taken as the standard of reference for other measures of tissue quantity.

The experiments reported in Table 2 and in Figs. 2 and 3 necessitated the speediest possible transfer and tissues could therefore be weighed only after incubation. In Table 2 and Fig. 2 results are quoted in terms of final tissue weight after incubation. These can be correlated with results based on fresh weight, since in the slices prepared by method (ii) the amount of fluid taken up during 30 min. incubation was 270 ± 20 mg./g. fresh wt. In Fig. 3 results with slices cut *in situ* (method iii) are quoted in terms of protein content and are compared with results from slices cut without fluid (method ii), which also have been expressed in terms of protein content. A factor for the conversion of μ equiv. of ion/g. fresh wt. to μ equiv. of ion/100 mg. of protein in the slices cut without fluid was obtained by measuring the protein content of six slices cut by the same method (ii), giving 97 ± 2 mg. of protein/g. fresh wt. after incubation. After incubation, tissue cut by method (iii) had 79 ± 4 mg. of protein/g. of incubated tissue, and the protein lost to the medium during incubation was 7.4 mg./g. of incubated tissue or 8.6% of the original protein. Slices cut with fluid (method i) and examined in the experiments shown in Figs. 4 and 5 had increased in weight, by absorption of fluid during preparation and before the initial weighing, to the extent of 20–25% (Varon & McIlwain, 1961). An increase of 23% was assumed in converting the results obtained by this method to a fresh-weight basis, as indicated in Fig. 4.

Fluid compartments and solute concentrations. Calculations for blood plasma and for tissue sampled *in situ*, and weighed and analysed without contact with fluid, followed those of Davson (1959), Woodbury (1955) and Cotlove, Holliday, Schwartz & Wallace (1951).

Blood plasma of the six animals sampled in the experiments of Table 4 gave values: Na^+ , 147 ± 1 mM; K^+ , 5.8 ± 0.3 mM; Cl^- , 104 ± 4 mM. The concentrations of these ions in extracellular fluid, resulting from a Gibbs–Donnan equilibrium, were calculated with factors given by Davson (1959): Na^+ , 139 mM; K^+ , 5.6 mM; Cl^- , 108 mM.

Fluid spaces in tissue prepared without contact with fluid were computed by assuming Cl^- ions to be extra-

cellular. The stages in calculating ion ratios in Table 4 are: from the fat content of the tissue, its water and ions were expressed on the basis of 1 g. of fat-free tissue. The volume occupied by Cl^- ions at the concentration computed for extracellular fluid (W_e^{Cl}) was derived ($425/108 = 394 \mu\text{l./g.}$ of fat-free tissue), and the intracellular fluid (W_i^{Cl}) obtained by difference from $861 \mu\text{l./g.}$ of fat-free tissue. Extracellular K^+ and Na^+ ions were calculated in the 1 g. of fat-free tissue (e.g. Na_e^{Cl} as $394 \mu\text{l.}$ at $139 \mu\text{equiv./ml.}$, giving $54.8 \mu\text{equiv./g.}$ of fat-free tissue) and the intracellular amounts obtained by difference (Na_i^{Cl} : $64.6 - 54.8 = 9.8 \mu\text{equiv./g.}$ of fat-free tissue). As a concentration in the intracellular water (which has been obtained by weight but is taken as having sp.gr. 1, the effects of other solids on both density and volume being ignored) this gives $[\text{Na}^+]_i$ as $9.8/467$, or 21.1 mM. The concentration ratio between Na^+ ions in the intracellular and extracellular fluids on the basis of chloride space is then $21.1/139$, or 0.15.

Fluid spaces in tissue (ii) which had been weighed before but analysed after incubation in fluids of known ion composition (Na^+ , 146; K^+ , 6.25; Cl^- , 135 mM) were obtained as follows. It was assumed that the increase in weight measured after draining represented increased water of sp.gr. 1, the total water associated with 1 g. of original tissue then being $800 + 270 = 1070$ mg. in a total weight of 1270 mg. or a fat-free wt. of 1152 mg. (original fat-free wt., 928 mg./g.). The ion content/g. original fat-free wt. was obtained from the observed values by the factor 0.928, giving e.g. Na^+ , $123/0.928 = 132 \mu\text{equiv./g.}$ of fat-free tissue and Cl^- , $86/0.928 = 92.6 \mu\text{equiv./g.}$ of fat-free tissue. The chloride space was then $92600/135 = 686 \mu\text{l./g.}$ of fat-free tissue, leaving a non-chloride space of $1151 - 686 = 465 \mu\text{l.}$ The chloride space then contained $146 \times 0.686 = 100 \mu\text{equiv.}$ of Na^+ /g. of fat-free tissue, giving an intracellular Na^+ concentration of $132 - 100 = 32/0.465$ or 68.5 mM, and a ratio to the external Na^+ of $68.5/146 = 0.47$.

Determination of fluid spaces in tissues (iii), which had been incubated before weighing, required knowledge of swelling during incubation; this was determined by rapidly weighing one set of slices, giving an increase in weight of 220 ± 30 mg./g. Subsequent calculations were as in the preceding paragraph. Fluid spaces in tissues (i) were computed similarly (Table 4) from data of Cummins & McIlwain (1961) and Varon & McIlwain (1961), taking the fluid uptake of 350 mg./g. from the latter paper.

RESULTS

Initial changes in sodium, chloride and potassium

When the brain was removed from guinea pigs and slices of cerebral cortex were cut from it without added fluids, placing the slices in fluid at 37° was followed by rapid movements of Na^+ , Cl^- and K^+ ions. Removing such slices at intervals from an oxygenated glucose-containing medium and draining them from adhering medium before analysis gave the results of Fig. 1.

The most rapid initial change is in Na^+ ions and occurs within 1 min., involving increase in Na^+ ions from a value of $61 \pm 2 \mu\text{equiv./g.}$ to $140 \pm 10 \mu\text{equiv./g.}$, or $79 \mu\text{equiv./g.}$ The concomitant changes in the other ions measured are: Cl^- , 28 ± 2 to 71 ± 2 ,

or $43 \mu\text{equiv./g.}$; K^+ , 114 ± 4 to 75 ± 2 , or $39 \mu\text{equiv./g.}$ Also, the tissue takes up fluid; direct weighing (see Experimental section) showed an increase of $270 \pm 20 \text{ mg./g.}$ of tissue. If this represents a gain in fluid of the composition of the medium, it would carry to each g. of tissue $39 \mu\text{equiv.}$ of Na^+ and $35 \mu\text{equiv.}$ of Cl^- . It thus appears that the increase in Na^+ ions (observed: $79 \mu\text{equiv.}$) can be accounted for by two processes: (i) an exchange of tissue K^+ ions for Na^+ ions, involving $39 \mu\text{equiv.}$ of each ion (the observed change in K^+ ions); (ii) uptake of fluid, carrying $39 \pm 3 \mu\text{equiv.}$ of Na^+ ions. The greater part of the change in Cl^- ions can also be ascribed to fluid uptake.

The tissue reaches approximate stability in its Na^+ , K^+ and Cl^- ions after 15–30 min. incubation and at this time its Na^+ ion content has increased by a further $20 \mu\text{equiv./g.}$ This is not associated with a net change in K^+ ion; but during this time the K^+ ion of the tissue has diminished, and then increased to its previous value (see Cummins & McIlwain, 1961). The changes in the three ions determined diverge further from a simple balance, for Cl^- ion has increased to $40 \mu\text{equiv./g.}$; however, this is understandable as several other relevant changes occur during incubation, including changes in tissue phosphates and lactate.

Fig. 1. Initial changes in the ion content of slices of guinea-pig cerebral cortex cut after removal from the body and without fluid (method ii). Slices were incubated for 30 min. in: $\circ$, $\square$, ∇ , bicarbonate-glucose medium; $\bullet$, $\blacksquare$, serum. Extensions from points: S.D. of six observations.

Attempts to minimize change in tissue sodium

Before examining electrically excited tissues, attempts were made to obtain tissue more normal in its Na^+ ion content by modifying the conditions under which it was prepared and incubated.

Incubation in guinea-pig serum rather than in medium did not modify the Na^+ or K^+ concentrations reached after 5 min.; values with whole serum are included in Fig. 1, and similar results were obtained with serum diluted 1:4 with the incubation medium. Other substances examined after longer periods of incubation are included in Table 1; creatine, guanosine and a group of bases with gangliosides were also without action (for the properties that led to examination of these materials, see Thomas, 1957; McIlwain, 1961a). Ascorbate, gangliosides and fraction IV-4 of blood plasma proteins increased the tissue Na^+ ions. Incubation of tissues in media low in Na^+ ions was found unsatisfactory, as described in a subsequent section.

It has, however, proved possible to minimize the change in tissue Na^+ ions by rapid preparation and incubation of the tissue. In exsanguinated animals the brain can be sliced *in situ* and the first slices placed in oxygenated media 1.5–2 min. after death. Also, the brain can be exposed in anaesthetized animals and slices obtained similarly promptly. After 30 min. incubation of tissues so prepared, in inulin-containing media, the tissues were drained, weighed and their Na^+ , K^+ and inulin determined. Fig. 2 summarizes these results and shows the time elapsing between death or the opening of the skull and the commencement of incubation to be the main factor that conditions the Na^+ and K^+ ions of the tissue. The two methods of obtaining the tissue gave equivalent results and anaesthesia is not therefore advantageous in this respect. Fig. 3 shows that the rapidly prepared tissue attains its lower Na^+ ion value by actual extrusion of Na^+ ions during the first 10 min. of incubation, a net rate of at least $180 \mu\text{equiv.}$ of Na^+ ions extruded/g. of tissue/hr. being reached during that time.

Electrically stimulated tissues

Electrical stimulation causes loss of K^+ ions from the tissue, the loss after a given time increasing with increasing intensity of stimulation (Fig. 4). Subsequent experiments employed the conditions of stimulation represented by 8–10 v peak potential in Fig. 4, and were thus appreciably but not greatly beyond the minimum needed for maximal movement of K^+ ions. In the tissue as ordinarily prepared and incubated and containing $65\text{--}70 \mu\text{equiv.}$ of K^+ ions/g. the maximal loss was of about $20 \mu\text{equiv./g.}$ About $30 \mu\text{equiv./g.}$ were lost from the tissue cut *in situ*, so that the K^+ ions of the two

Table 1. Ion content of cerebral cortex after incubation in modified media

The cerebral cortex was removed from guinea pigs, cut without fluid, weighed and incubated in glucose-bicarbonate medium with the additions described for 30 min. Values are given $\pm$ s.d. when appropriate.

Substance added to medium (mm unless otherwise stated)	Composition after incubation (μ equiv./g. of fresh tissue)				No. of slices
	K ⁺	Total Na ⁺	Na ⁺ of non-inulin space	Cl ⁻	
None	83 $\pm$ 1	156 $\pm$ 4	74 $\pm$ 4	109 $\pm$ 5	12
Ganglioside (0.2 mg./ml.)	78 $\pm$ 1	186 $\pm$ 3	90 $\pm$ 1	110 $\pm$ 2	5
Serum proteins IV-4 (10 mg./ml.)	80	186	91	—	2
Ascorbate	79 $\pm$ 2	176 $\pm$ 3	86 $\pm$ 2	112 $\pm$ 2	5
Adenosine	81 $\pm$ 2	157 $\pm$ 3	77 $\pm$ 2	109 $\pm$ 2	5
Creatine	78	153	71	117	2
Guanosine	78	151	72	115	2
Adenosine, creatine and guanosine	79	174	75	—	3
Adenosine, creatine, guanosine and ganglioside (0.2 mg./ml.)	77 $\pm$ 3	158 $\pm$ 3	77 $\pm$ 2	100 $\pm$ 3	4

Fig. 2. The Na⁺ and K⁺ ions of guinea-pig cerebral cortex out *in situ* (method iii) and incubated for 30 min., incubation commencing at intervals of 2–9 min. after the skull was opened. O, Anaesthetized animals; ●, exsanguinated animals. Extensions from points: s.d. of six observations. Left-hand scale: observed values. Right-hand scale: values calculated to fresh wt. (see Experimental section). The line and the equation of the line were obtained by regression analysis; numbers refer to the left-hand scale.

Fig. 3. The Na⁺ and K⁺ ions of guinea-pig cerebral cortex out before (●) and after (○) removal of the brain from the animal (methods iii and ii), and subsequently incubated in glucose-bicarbonate medium. Extensions from points: s.d. of six observations. Left-hand scale: observed values. Right-hand scales: values calculated to fresh wt. (see Experimental section).

groups of tissues after stimulation were alike (Table 2). Concomitantly with the loss in K⁺ ions, the tissues gained about an equivalent of Na⁺ ions. When pulses were stopped, these ion movements were reversed (Table 2). Potassium, as previously found, re-entered the tissue and Na⁺ ion was then observed to be extruded. The maximum rates of extrusion observed were 180–240 μ equiv. of Na⁺ ions/g. of tissue/hr. The net movements in Na⁺ and

K⁺ ions were about equivalent, but greatly slowed when only about 1/3 to 1/2 of the ions displaced had been recovered.

Potassium and the excitability of tissues
in media low in sodium salts

Reciprocal relationships between tissue K⁺ and Na⁺ ions were observed also when slices were in-

incubated in media with little or no Na^+ ions. Choline chloride or bicarbonate or tris hydrochloride was used to replace Na^+ ions in the experiments of Table 3. Whereas these readily yielded tissues low in Na^+ ions, tissue K^+ ion also was depressed, in agreement with observations of Pappius, Rosenfeld, Johnson & Elliott (1958) and Hillman & McIlwain (1961). It was then attempted to restore tissue K^+ but not Na^+ ions by returning the low- Na^+ ion tissue to media of normal composition. This was not, however, successful (Table 3): tissue Na^+ ion was increased to a greater extent than tissue K^+ ion.

The linked movements of sodium and potassium salts implied by these findings were further explored by incubating slices in media containing a

range of Na^+ ion concentrations and measuring respiration both with and without the application of electrical pulses. Immediately after application of pulses the tissues were briefly rinsed in a cold iso-osmotic sucrose solution and their Na^+ and K^+ ions determined. Under these conditions also, limiting the Na^+ ions diminished tissue K^+ ions and about 100 mM- Na^+ ion was needed in the incubating fluid to give normal K^+ ion values (45–50 μ -equiv./g., after electrical stimulation: Fig. 5). Concomitantly, the diminution in Na^+ ions limited the change in tissue respiration caused by electrical pulses (Fig. 5). This had been noted to occur in a previous study (Gore & McIlwain, 1952), in which, however, two concentrations only of sodium salts were examined. The present results show that at least 100 mM- Na^+ ion was needed for maximal respiratory response, as was also required for K^+ ion maintenance.

DISCUSSION

Tissue sodium and sodium movements

Data on the distribution of sodium between cerebral tissues and extracellular fluids are given in Table 4; in calculating these data, two approximations to the cellular space of the tissue have been used. (i) The first assumes that the extracellular part of the tissue has the sodium and the chloride content of the extracellular fluid, and that there is no chloride intracellularly; probably some chloride exists in the cells (see Varon & McIlwain, 1961) and therefore these ratios are too low. (ii) The second assumes a similar distribution for inulin, though inulin probably does not penetrate all extracellular spaces accessible to sodium or chloride, and thus the values are too high.

In vivo, values for the ratio based on chloride space are available, but none based on inulin which is excluded from the brain. As ordinarily prepared by cutting in aqueous solutions (method i: Experimental section) much additional fluid and sodium

Fig. 4. Intensity of electrical stimulation and the potassium content of cerebral cortex. Slices prepared by method (i) were incubated for 60 min., during the last 30 min. of which condenser pulses of exponential time-voltage relationships were applied, with time-constant 0.4 msec., at 100/sec. and of the peak potential quoted. The mean initial K^+ ion concentration was 57 μ equiv./g. of wet tissue, or 70 μ equiv./g. of fresh tissue; final K^+ ion concentrations, 39 or 48 μ equiv./g. Points give the mean values of a minimum of six values and lines extend from the points to distances equal to the s.d.

Table 2. Sodium and potassium of electrically stimulated tissues

After preincubation of slices in quick-transfer electrodes for 30 min. in glucose-bicarbonate medium with inulin, electrical pulses of peak potential 10v and the other characteristics of Fig. 4 were applied for 10 min. Slices were then drained on glass, weighed and transferred to trichloroacetic acid either immediately or after recovery periods of 2 or 5 min. Contents quoted are mean values $\pm$ s.d. with the number of observations in parentheses.

		Content (μ equiv./g. final wt.)			
Ion measured	Preparation of tissue	No pulses	With pulses		
			No recovery	Recovery for 2 min.	Recovery for 5 min.
K ⁺	Cut <i>in situ</i>	76 $\pm$ 2 (12)	45 $\pm$ 2 (12)	53 $\pm$ 1 (8)	56 $\pm$ 1 (8)
	Removed and cut	65 $\pm$ 1 (12)	42 $\pm$ 1 (18)	50 $\pm$ 1 (18)	53 $\pm$ 1 (6)
Na ⁺ of non-inulin space	Cut <i>in situ</i>	34 $\pm$ 3 (12)	62 $\pm$ 3 (12)	58 $\pm$ 3 (8)	49 $\pm$ 3 (8)
	Removed and cut	58 $\pm$ 4 (12)	87 $\pm$ 3 (18)	79 $\pm$ 3 (18)	80 $\pm$ 4 (6)

Table 3. Incubation of media diminished in sodium

Changes in medium			Tissue composition					No. of slices	
NaCl replaced by	NaHCO ₃ replaced by	Na ⁺ of medium (mm)	K ⁺ (μequiv./g.)	Total Na ⁺ (μequiv./g.)	Na ⁺ in non-inulin space (μequiv./g.)	Total Cl ⁻ (μequiv./g.)	Cl in non-inulin space (μequiv./g.)	Swelling (μl./100 mg.)	Inulin space (μl./100 mg.)
No change	No change	146	83 ± 1	156 ± 6	74 ± 4	110 ± 4	39 ± 3	27 ± 3	55 ± 2
Choline-Cl	Choline-HCO ₃	0	26 ± 1	12 ± 3	12 ± 3	154 ± 6	78 ± 4	30 ± 2	57 ± 3
Choline-Cl	No change	30	51 ± 1	35 ± 1	18 ± 1	104 ± 4	37 ± 3	20 ± 1	53 ± 2
*Choline-Cl	No change	30, then 146	58 ± 2	128 ± 2	50 ± 4	110 ± 2	45 ± 4	29 ± 1	50 ± 2
Tris-HCl	No change	30	42 ± 1	34 ± 1	10 ± 1	58 ± 1	38 ± 1	26 ± 4	33 ± 3
*Tris-HCl	No change	30, then 146	44 ± 1	174 ± 7	69 ± 4	105 ± 7	48 ± 5	40 ± 2	44 ± 2

Slices, prepared after removal of the brain, were incubated in the rapid-transfer apparatus for 30 min. in glucose-bicarbonate medium in which NaCl and NaHCO₃ had been replaced by choline or tris salts. In certain experiments, marked by asterisks, slices were preincubated for 20 min. in the low Na⁺ ion medium and were then transferred to media of the normal Na⁺ ion content for a further 30 min. Weights refer to the fresh tissue and mean results (six slices) are given ± s.d.

Fig. 5. Guinea-pig cerebral cortex slices were incubated in grid electrodes in manometric vessels containing media of the Na⁺ ion content noted; tris-HCl replaced the NaCl. After 30 min. incubation condenser pulses of 10v peak potential, and the other characteristics of Fig. 4, were applied for a further 30 min. Points give the mean values of 6-12 observations and lines extend from the points to distances equal to the s.d.

have become attached to the tissue and the ratio of intracellular to extracellular sodium concentrations has greatly increased. This increase is diminished by preparation of the slices in the absence of added fluid (method ii), but remains three times as great as *in vivo*. Cutting the tissue *in situ* and its transfer to incubating solutions within 2 min. of cutting, however, diminishes the ratio still further and it now comes, at 0.36, closer to that of the tissue *in vivo*. Values based on non-inulin space show a corresponding fall.

That the sodium of tissue prepared by method (iii) is lower is not due to the method of preparation giving less time for entry of sodium: sodium enters but is actively extruded (Fig. 3), whereas extrusion does not occur in tissue prepared by method (ii). Nevertheless, tissue prepared by either method is capable of extruding sodium when its sodium has been increased by electrical stimulation (Table 2); though in neither case is the sodium recovery extensive enough to diminish the tissue sodium to its value before stimulation. Tissue mechanisms

concerned with movement of sodium thus appear relatively labile, and to merit examination by study of the several component processes individually by sodium isotopes. It is satisfactory that extrusion of sodium, though partial, includes periods when its net velocity is at 180–240 $\mu\text{equiv./g.}$ of tissue/hr., or higher.

Tissue potassium and other aspects of tissue metabolism

The rapid method of tissue preparation, devised to minimize uptake of sodium, yielded specimens of higher potassium content than otherwise obtained; reciprocal relations between the two ions are shown in Fig. 2. Although this method resulted in tissue closer in its potassium content to that of the tissue *in vivo*, the values *in vivo* were not reached (Table 4): neither when appraised by content per unit weight nor by ratio of potassium concentrations computed for intracellular and extracellular fluids.

Active processes in the maintenance of tissue potassium were nevertheless clearly shown in the present system, rapid net assimilation being

observed both at the beginning of incubation and also after applying pulses. Moreover, potassium maintenance required sodium, as previously indicated in cerebral tissues by Pappius *et al.* (1958), and also by Hillman & McIlwain (1961) during a study of the cellular membrane potentials of the tissue, potentials which themselves required sodium and potassium for maintenance. Requirement of sodium for maintenance or assimilation of cellular potassium has now been shown in several systems, since recognition of linkage between movements of the two ions in erythrocytes by Harris & Maizels (1951). Glynn's (1962) observations with erythrocytes show especially clearly that utilization of adenosine triphosphate in maintaining potassium requires both sodium and potassium. Evidence for the involvement of a sodium- and potassium-activated adenosine triphosphatase in the present tissue has been given by Deul & McIlwain (1961*a, b*) and A. Schwartz, H. S. Bachelard & H. McIlwain (unpublished work). Such a system would be expected to transport less potassium in the absence of sodium, and to yield less inorganic phosphate and adenosine diphos-

Table 4. *Computed tissue spaces and ion concentrations in guinea-pig cerebral cortex*

For sources and derivation of data, see Experimental section.

Method of preparation of tissue	...	(ii)	(i)*	(i)	(ii)	(iii)
Measurement						
No. of specimens	...	6	4–6	6	24	12
Subsequent treatment	...	None	Incubated	Incubated	Incubated	Incubated
Basis						
	...	Fresh tissue	Incubated tissue	Incubated tissue	Incubated tissue	Incubated tissue
Observed						
Cl ⁻ ($\mu\text{equiv./g.}$)		39.5 $\pm$ 5.5	74 $\pm$ 5	83 $\pm$ 4	86 $\pm$ 5	74 $\pm$ 7
Na ⁺ ($\mu\text{equiv./g.}$)		60.0 $\pm$ 4.0	121 $\pm$ 5	120 $\pm$ 4	123 $\pm$ 4	105 $\pm$ 3
K ⁺ ($\mu\text{equiv./g.}$)		112.0 $\pm$ 4.5	53 $\pm$ 5	62 $\pm$ 3	65 $\pm$ 1	77 $\pm$ 2
Inulin space ($\mu\text{l./g.}$)		—	—	500 $\pm$ 30	434 $\pm$ 20	487 $\pm$ 25
Uptake of fluid ($\mu\text{l./g.}$)		—	270†	265 $\pm$ 40	270 $\pm$ 20	220 $\pm$ 30
Calculated [on basis of fat-free tissue ($\mu\text{l.}$ or $\mu\text{equiv./g.}$ of fat-free tissue)]						
Cl ⁻ ($\mu\text{equiv./g.}$)		42.5	79.6	89.5	92.6	79.6
Cl ⁻ space ($\mu\text{l./g.}$)		394	590	664	686	590
Inulin space ($\mu\text{l./g.}$)		—	—	539	467	525
Water (mg./g.)		861	1151	1147	1151	1097
Non-Cl ⁻ space ($\mu\text{l./g.}$)		467	561	483	465	507
Non-inulin space ($\mu\text{l./g.}$)		—	—	608	684	572
K ⁺ ($\mu\text{equiv./g.}$)		119.3	57	67	70	83
K ⁺ ($\mu\text{equiv./Cl- space}$)		2.2	3.7	4.2	4.3	3.7
K ⁺ of non-Cl ⁻ space (mm)		250	95	130	141	157
K ⁺ ($\mu\text{equiv./inulin space}$)		—	—	3.3	2.9	3.3
K ⁺ of non-inulin space (mm)		—	—	105	98	138
Ratio (K ⁺ , non-Cl ⁻)/(K ⁺ , ext.)		44.7 $\pm$ 3.0	15.2	20.6 $\pm$ 3.5	22.4 $\pm$ 3.8	24.9 $\pm$ 1.9
Ratio (K ⁺ , non-inulin)/(K ⁺ , ext.)		—	—	17.1 $\pm$ 2.0	16.3 $\pm$ 3.2	22.6 $\pm$ 3.3
Na ⁺ ($\mu\text{equiv./g.}$)		64.6	130	129	132	113
Na ⁺ ($\mu\text{equiv./Cl- space}$)		54.8	86	96.5	100	86
Na ⁺ of non-Cl ⁻ space (mm)		21.1	79	67	68.5	53.1
Na ⁺ ($\mu\text{equiv./inulin space}$)		—	—	78.5	68	76.5
Na ⁺ of non-inulin space (mm)		—	—	84	96	65
Ratio (Na ⁺ , non-Cl ⁻)/(Na ⁺ , ext.)		0.15 $\pm$ 0.06	0.54	0.49 $\pm$ 0.11	0.46 $\pm$ 0.09	0.36 $\pm$ 0.08
Ratio (Na ⁺ , non-inulin)/(Na ⁺ , ext.)		—	—	0.56 $\pm$ 0.09	0.63 $\pm$ 0.10	0.46 $\pm$ 0.06

* Data from Cummins & McIlwain (1961) except value marked †.

† Value from experiments subsequent to Cummins & McIlwain (1961).

phate: the lesser quantities of phosphates give the basis for lesser respiratory response to pulses in the absence of sodium (see McIlwain, 1952), as observed in Fig. 5.

SUMMARY

1. Cerebral tissues incubated in oxygenated glucose-bicarbonate media rapidly gained sodium: about 40 μ equiv. of sodium/g. by exchange with potassium and a similar quantity by uptake of sodium chloride, mainly during the first minute's contact with the media.

2. Attempts were made to minimize these changes by alterations in the incubating medium. A number of added substances were without such effect; preincubation in media low in sodium did not lead to lower tissue sodium when, subsequently, slices were placed in media of normal sodium content.

3. Rapid preparation of tissue by cutting it *in situ* yielded samples of lower sodium after incubation; during incubation, a net extrusion of sodium was observed in such samples, at rates of 180–240 μ equiv./g. of tissue/hr.

4. Electrical stimulation increased the sodium content of the incubated tissue; after cessation of stimulation, the additional sodium was in part extruded, again at about 200 μ equiv./g. of tissue/hr.

5. Tissue incubated in media low in sodium content, between 0 and 75 mM, was low in potassium content; when stimulated electrically, the respiratory rate of such tissues changed by –50 to +60% rather than by the +100% shown in media of normal sodium content.

6. The intracellular to extracellular ratios of Na^+ and K^+ ions (based on chloride and inulin spaces) have been calculated for incubated tissues

prepared by different techniques and compared with the ratios (based on chloride space) in unincubated fresh tissue.

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Gangliosides, Phospholipids, Protein and Ribonucleic Acid in Subfractions of Cerebral Microsomal Material

By J. R. WHERRETT AND H. McILWAIN

Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, London, S.E. 5

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Potassium movements and excitability in cerebral tissues that have been kept in fluid at 0° are altered by added gangliosides; much of the tissues' native gangliosides is found in microsomal material (McIlwain, Woodman & Cummins, 1961; McIlwain, 1961; Wolfe, 1961). Excitability and

ion movements are likely to be membrane phenomena and the microsome fraction contains fragments of membranes, but it also contains other constituents, notably ribonucleic acid granules (Hanson & Toschi, 1959, 1960). Further knowledge of the subcellular localization of gangliosides can