Therefore when $t > 2\frac{1}{2}$ hr., each $1 - \exp[-bt]$ term in equation for X_4 is equal to 1, bt or 0. At least one $1 - \exp[-bt]$ term is equal to 1 and at least one $1 - \exp[-bt]$ term = bt . Therefore if $b_2 \sim K_{34}$, both $1 - \exp[-b_2t]$ and $1 - \exp[-K_{34}t]$ are equal to 1. The only alternative is for $b_2 \gg K_{34}$ or $K_{34} \gg b_2$. If $b_2 \gg K_{34}$ the intercept is negative, and if $K_{34} \gg b_2$ the slope is zero, and so neither of these alternatives is possible.

$$\therefore X_4 \simeq \frac{K_{12}K_{23}}{K_{21}+K_{23}}t - \frac{K_{12}K_{23}}{(K_{21}+K_{23})} \left(\frac{1}{K_{34}} + \frac{1}{K_{21}+K_{23}} \right)$$

$$\therefore S = \frac{K_{12}K_{23}}{K_{21}+K_{23}} = \frac{K_{23}M_2}{M_1} \quad (1)$$

$$\text{and} \quad t_0 = \frac{1}{K_{34}} + \frac{1}{K_{21}+K_{23}}. \quad (2)$$

$$\text{Similarly,} \quad X_3 \simeq \frac{K_{23}M_2}{K_{34}M_1} \quad (3)$$

$$\text{and} \quad X_2 = \frac{M_2}{M_1}. \quad (4)$$

The equations (a)–(f) given in the Methods section are readily obtained from equations (1)–(4).

Equations (1)–(4) may be solved to obtain values for the four unknowns, K_{12} , K_{23} , K_{21} , K_{34} (and hence M_2/M_1) in terms of S , t_0 , X_3 and X_2 . However, these results have not been used as it was considered that the absolute experimental results

were not sufficiently accurate. Only ratios of values of S , t_0 , X_3 and X_2 for albumin to the same values for γ -globulin have been used.

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The Dependence of the Respiration of Brain Cortex on Active Cation Transport

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The widespread dependence upon respiration of the maintenance of concentration gradients of sodium and potassium between cells and plasma has been established during the past decade, the notable exception being mammalian erythrocytes, which depend on glycolysis. The work of Turner, Eggleston & Krebs (1950) and of Krebs, Eggleston & Turner (1951), for example, showed that brain-cortex slices lose potassium when respiration is inhibited with respiratory poisons and, further, that the exchange of potassium between cells and medium is at the high rate of about 4 m-equiv./kg.

wet wt./min., representing the passive outward leakage which is made good by an active influx. The loss of potassium caused by 2,4-dinitrophenol suggested that active transport did not depend directly upon respiration as such but upon a supply of adenosine triphosphate, the utilization of which is inhibited by this compound.

More recent experiments with other cells have shown unequivocally that adenosine triphosphate facilitates active movements of Na^+ and K^+ ions. Thus the efflux of sodium from squid giant axons is inhibited by cyanide, yet the injection into poisoned

axons of adenosine triphosphate or compounds that generate adenosine triphosphate (e.g. arginine phosphate) restores the sodium efflux (Caldwell, Hodgkin, Keynes & Shaw, 1960). Again, the introduction of adenosine triphosphate into erythrocyte ghosts at the time of haemolysis, by the technique of the reversal of haemolysis, promotes the accumulation of potassium (Gardos, 1954; Whittam, 1957) and the extrusion of sodium (Hoffman, 1960) that does not otherwise occur during subsequent incubation. Rather more indirect evidence with erythrocytes (Dunham, 1957; Whittam, 1958), ascites-tumour cells (Maizels, Remington & Truscove, 1958), seminal-vesicle-mucosa slices (Whittam & Breuer, 1959) and kidney slices from newborn animals (Whittam, 1960) gives further support for the view that adenosine triphosphate is required for active cation transport, the dependence of which on metabolism is thus readily explained.

The role of adenosine triphosphate as at least one of the links between metabolism and active transport suggests the possibility that the two processes may be tightly coupled. It is known that mitochondrial respiration is largely under the control of adenosine diphosphate through processes utilizing adenosine triphosphate, such as muscular contraction, or through the hydrolysis of adenosine triphosphate by adenosine triphosphatase (Chance & Williams, 1955), and the question arises whether part of a cell's respiration is controlled by the fission of adenosine triphosphate caused by the active transport mechanism. This is the converse of the dependence of active transport upon respiration, and the aim of the present work has been to find out if a failure to maintain a high potassium concentration in brain-cortex slices, induced by specific means that do not directly inhibit respiration, is accompanied, nonetheless, by a fall in the rate of respiration. Brain cortex was selected as a suitable tissue because both the rate of respiration and the rate of exchange of potassium are high, the latter making it possible to measure net falls in the tissue potassium concentration when the active

influx is inhibited. A comparison has been made between the tissue potassium concentration and the rate of respiration after incubation under various conditions for 1 hr. The results show that about 40 % of the respiration appears to be coupled to an active transport system for potassium, which therefore acts as a pacemaker of respiration.

METHODS

Adult rabbits were stunned by a blow on the head, bled from the neck and decapitated. The brain was excised and the medulla cut away from the cortex, which was then immediately sliced or homogenized. Slices were cut free-hand by the method of Deutsch (1936), with a razor blade moistened with medium. The slices were placed in the medium in which they were to be incubated in a Petri dish at room temperature until they had all been cut. About 50 mg. of tissue (wet wt.) was transferred to 4.0 ml. of medium in cups of Warburg manometers, in which the O_2 consumption was measured during incubation for 1 hr. at 37°.

A sample of tissue at the start of incubation and all the incubated samples were rapidly blotted on hardened filter paper (Whatman no. 542) and the dry weight was determined by weighing after drying the tissues in tared beakers during the night at 105°.

The whole of the brain cortex (about 5 g.) was homogenized in a glass tube (capacity 20 ml.; internal diam. 1.3 cm.) containing a tightly fitting plunger, the standard procedure being to raise the tube containing the tissue plus medium (occupying about 8 ml.) three times to cause disintegration of the tissue by the rotating plunger (700 rev./min.). The homogenate was strained through muslin and the volume made to 14–15 ml. by the addition of more medium. Portions of homogenate (1.0 ml.) were then added to manometer cups and the O_2 consumption was measured during incubation at 37°. (Q_{O_2} = μ l. of O_2 /mg. dry wt./hr.)

Media. The composition of the standard media is shown in Table 1. In order to obtain values for the sodium concentration between 0 and 150 mM, appropriate amounts of choline chloride, KCl or sucrose were replaced by NaCl solution.

Analytical methods. Sodium and potassium were estimated by flame photometry (Amoore, Parsons & Werkheiser, 1958) on dried samples of tissue, which were digested in conc. HNO_3 as described by Whittam & Davies (1953).

Table 1. *Composition of media*

Media were prepared with the compositions shown below, and slices and homogenates were incubated therein.

Kind of medium ...	Sodium-rich		Sodium-free	
	NaCl	Choline chloride	Sucrose	KCl
Main solute ...	0.15 M-NaCl	0.15 M-Choline chloride	0.25 M-Sucrose	0.15 M-KCl
	5 mM-KCl	—	—	—
	10 mM-Sodium phosphate, pH 7.4	2.5 mM-Potassium phosphate, pH 7.4	2.5 mM-Potassium phosphate, pH 7.4	2.5 mM-Potassium phosphate, pH 7.4
	2 mM-MgCl ₂	2 mM-MgCl ₂	2 mM-MgCl ₂	2 mM-MgCl ₂
	10 mM-Glucose	10 mM-Glucose	10 mM-Glucose	10 mM-Glucose

RESULTS

Potassium concentration in slices

Effect of ouabain. The work of Schatzmann (1953) and others (see Glynn, 1957) has shown that cardiac glycosides, of which ouabain is the most potent, inhibit the active uptake of potassium by human erythrocytes without affecting glycolysis. In order to find out whether the uptake and the retention of potassium in respiring brain-cortex slices was also prevented by ouabain, slices were incubated for 1 hr. at 37° in NaCl medium (Table 1) containing glucose and different concentrations of ouabain and then analysed for potassium.

The potassium concentration (m-equiv./kg. dry wt.) in the tissue was 275 ± 20 (19) before incubation and 361 ± 29 (6) after incubation of the control without ouabain, indicating a net uptake of 86 ± 35 . The potassium concentration is plotted against the ouabain concentration in Fig. 1 and shows that a net loss of potassium from tissue to medium occurred with ouabain concentrations of $0.5 \mu\text{M}$ or larger. Thus $2.5 \mu\text{M}$ -ouabain caused a fall in the concentration to 118 ± 13 (3) m-equiv./kg. dry wt. and, when the effect of ouabain was maximal, the tissue contained about 100 m-equiv./kg. dry wt., which is 70 % less than the potassium

in the control. The shape of the curve in Fig. 1 suggests that the fall in potassium concentration might be an exponential function of the ouabain concentration, and this was shown to be true by the straight line obtained when the logarithm of the ratio

$$\frac{[\text{K}] \text{ in control} - [\text{K}] \text{ with max. inhibition}}{[\text{K}] \text{ with ouabain} - [\text{K}] \text{ with max. inhibition}}$$

was plotted against the ouabain concentration. The ouabain concentration required for half-maximal effect was about $0.4 \mu\text{M}$. The results show that the uptake and retention of potassium by the tissue during incubation in a medium containing glucose was inhibited by low concentrations of ouabain, which caused an exponential fall in the potassium concentration of the tissue.

Turner *et al.* (1950) noted that more potassium was accumulated by brain-cortex slices during incubation with glutamate than with glucose and it was of interest to see if this extra uptake was also prevented by ouabain. The potassium concentration (m-equiv./kg. dry wt.) of 460 in the presence of glutamate fell to about 100 with $2.5 \mu\text{M}$ or larger concentrations of ouabain. The mean concentration found with glucose plus glutamate was 492 ± 42 (6) whereas with glucose alone it was 360. Low concentrations of ouabain again caused a loss of tissue potassium to the medium and the final concentration of 100 m-equiv./kg. dry wt. was the same as that in slices incubated with glucose alone. Further, the ouabain concentration in the medium containing glutamate needed to cause a half-maximal loss of tissue potassium was also about $0.5 \mu\text{M}$. Since the addition of glutamate did not overcome the effect of ouabain, the rest of the experiments were made with media containing just glucose as substrate. This was an adequate substrate for the uptake and retention of tissue potassium, although not of the extra amount associated with glutamate (Stern, Eggleston, Hems & Krebs, 1949).

The sodium concentration in the tissue was not determined, because the large amount of extracellular sodium makes changes in the total tissue concentration a less accurate indication of active transport than changes in the potassium concentration. The extracellular space was also not measured, as it does not need to be known accurately to show that the bulk of the tissue potassium must have been intracellular. Thus 98 % of the tissue potassium would be intracellular even assuming an extracellular space of 30 % with a potassium concentration equal to that of the medium (5 mM). The changes in the tissue potassium concentration described above therefore represent changes in the intracellular content and indicate whether active transport of potassium was

Fig. 1. Fall in potassium concentration of brain-cortex slices after incubation for 1 hr. at 37° with ouabain. Slices were incubated in 4.0 ml. of NaCl medium (see Table 1) containing different concentrations of ouabain in Warburg vessels gassed with O_2 . The s.e.m. is indicated by the vertical lines through the points and the numbers of observations are stated in parentheses. The potassium concentration of the tissue before incubation was 275 m-equiv./kg. dry wt. and the change in concentration during incubation is indicated by the right-hand ordinate.

occurring across the cell membranes. The tissue water content was also not always determined but in a few experiments it was measured as about 85% (w/w). The potassium concentration in the control tissue was therefore about 50 m-equiv./kg. wet wt., or 60 m-equiv./kg. of tissue water, which may be compared with a concentration in the medium of 5 mM.

Effect of sodium-free media. The uptake of potassium may be a primary active process independent of the simultaneous extrusion of sodium or it may occur only consequentially when sodium is being pumped out. In the latter case, the potassium uptake would depend upon the presence of a concentration gradient of sodium between intra- and extra-cellular fluid. The replacement of sodium of the medium with choline would therefore be expected to stop the active influx of potassium that is coupled to sodium efflux, because in the absence of external sodium the concentration gradient would disappear and the tissue sodium would quickly leak out passively, leaving none to be pumped out. Slices were allowed to remain in the sodium-free medium in a Petri dish at room temperature until they had all been made, when they were incubated for 1 hr. at 37° in choline chloride medium in which the potassium concentration was the same (5 mM) as in the sodium-rich medium, and then analysed for sodium and potassium. The tissue sodium concentration fell to the very low value of about 25 m-equiv./kg. dry wt., and the potassium concentration also fell to the low value of 108 m-equiv./kg. dry wt., which is to be compared with the value of 360 m-equiv./kg. dry wt. found after incubation in the sodium-rich (150 mM) medium. The replacement of sodium of the medium with choline therefore caused a fall in the tissue potassium concentration to the same as that found after incubation in sodium-rich medium containing 2.5 μ M-ouabain (Fig. 1). Furthermore, incubation in sodium-free medium containing 5 μ M-ouabain caused no further fall in the potassium concentration; the mean value in four experiments was 105 m-equiv./kg. dry wt. The amount of potassium found in the tissue after abolition of active transport, either by ouabain in NaCl medium or by the replacement of sodium by choline, is equal to that which Folch, Lees & Sloane-Stanley (1957) found to be bound by phospholipids and phosphatidylserine when these compounds were extracted from brain by organic solvents. The results show: first, that the removal of sodium from the medium caused a loss of tissue potassium, the retention of which would therefore normally seem to depend upon sodium extrusion; secondly, that ouabain had no effect on the low tissue potassium concentration found after incubation in sodium-free medium. The leakage of both sodium and

potassium from the tissue in choline chloride medium would leave the intracellular fluid hypo-osmotic unless water and chloride accompanied the cations or unless choline entered the cells to balance the loss. The loss of tissue chloride and water would lower the percentage water of the tissue and, as this was the same in choline chloride and NaCl medium (85–86%), it would seem that choline entered the cells in replacement of the sodium and potassium that leaked out.

Respiration of brain slices

In order to find out if the stoppage of active transport by ouabain or the removal of sodium was accompanied by a fall in Q_{O_2} , the consumption of O_2 was measured. The uptake of O_2 of brain-cortex slices was depressed by concentrations of ouabain sufficient to inhibit the active transport of potassium, about 50% inhibition being produced with 5 μ M when the Q_{O_2} fell from 16.7 ± 0.7 to 8.0 ± 0.7 (3) (Fig. 2). The results did not give a straight line with a semi-logarithmic plot, probably because of the scatter of the results of different experiments, and the concentration required to produce half-maximal inhibition can be given only roughly as 2 μ M-ouabain.

The Q_{O_2} of slices incubated in (sodium-free) choline chloride medium was 9.4 ± 0.4 (7), which was not further reduced during incubation with 10 μ M-ouabain (Q_{O_2} 8.8). These values are similar to those found for slices incubated in NaCl medium containing 5 μ M-ouabain. When the NaCl of the medium was replaced with sucrose, the Q_{O_2} (5.7)

Fig. 2. Fall in Q_{O_2} of brain-cortex slices in the presence of ouabain. Conditions were as in Fig. 1. The s.e.m. is indicated by the vertical straight lines and bars and the numbers of observations are given in parentheses.

was again unchanged with $10\text{ }\mu\text{M}$ -ouabain. These values are lower than those of slices in choline chloride medium because of the extra dry weight of the sucrose, a correction for which was not made. Since these results mean that respiration was stimulated by sodium, it was interesting to see how much was required to activate respiration. The sodium concentration required to produce half-maximal stimulation was found by incubating slices in media of different sodium concentrations, and Fig. 3 shows that it was $15\text{--}20\text{ mM}$, irrespective of whether Na^+ ions were replaced by choline or by sucrose. The addition of ouabain in the sodium-rich medium and the removal of sodium from the medium thus cause comparable falls in the Q_{O_2} of slices and, in the absence of sodium in the medium, ouabain had no effect on Q_{O_2} .

Respiration of brain homogenates

The sensitivity of the respiration of slices to ouabain and sodium suggests that one or more pace-maker reactions hydrolysing ATP to ADP were inhibited and activated respectively. It was therefore necessary to see if the respiration of homogenates was also similarly affected by ouabain and the deprivation of sodium.

No attempt has been made to fractionate the whole homogenates, which were made in solutions selected so as to include sodium (0.15 M -NaCl) or to exclude sodium (0.25 M -sucrose; 0.15 M -KCl; 0.15 M -

choline chloride). A sample of each homogenate (1.0 ml.) was added to 3.0 ml. of medium for incubation. Each homogenate was incubated with medium containing sodium, and homogenates made in sodium-free solutions were also incubated in the same (sodium-free) solution in which they were made. Thus a sample (1.0 ml.) of sodium-free homogenate added to 3.0 ml. of medium containing 0.15 M -NaCl yielded a mixture for incubation containing 112 mM -NaCl, the respiration of which was compared with that of sodium-free mixtures to find the effect of sodium. Further, both the sodium-free and the sodium-rich preparations were incubated with $5\text{ }\mu\text{M}$ -ouabain, in order to find if the inhibitory effect of ouabain depended on the presence of sodium in the medium.

Effect of ouabain. The results (Table 2) show that in the sodium-rich media Q_{O_2} was $2.33\text{--}2.70$, irrespective of whether the homogenate was made in NaCl, KCl or choline chloride. The addition of $5\text{ }\mu\text{M}$ -ouabain caused a fall of about 40%, to $1.40\text{--}1.69$. The dry weight of the sucrose homogenate was not corrected for the weight of sucrose, and although the Q_{O_2} values are correspondingly lower they still show a similar reduction of 34% with ouabain (from 1.65 to 1.09). In a medium containing 112 or 150 mM -NaCl, the Q_{O_2} of homogenates was therefore reduced by ouabain in the same way as that of tissue slices.

Effect of sodium. The rates of respiration of sodium-free homogenates were about 40% lower than those of homogenates containing 112 mM -NaCl, and they were not reduced further by the presence of ouabain (Table 2). These results show that, as was found with slices, the inhibitory effect of ouabain on the rate of respiration was manifest only when sodium was present in the medium, and that sodium stimulated respiration.

There is some spread of results in Table 2, but the value of the ratio: fall in Q_{O_2} with omission of sodium from medium/fall in Q_{O_2} with ouabain present in a sodium-rich medium, is close to unity. The values, 1.07 ± 0.14 , 1.21 ± 0.27 and 1.02 ± 0.16 for homogenates made in KCl, choline chloride and sucrose solutions respectively, show that the falls in Q_{O_2} caused by the addition of ouabain in sodium-rich medium and by the deprivation of sodium are the same.

Half-maximal stimulation by sodium. The increase in Q_{O_2} on the addition of sodium to the medium made it worth while to find the concentration of sodium required to produce half-maximal stimulation. Homogenates were therefore made in choline chloride and in sucrose media. Samples (1.0 ml.) were incubated with volumes of NaCl medium such that the range in the concentration of the added sodium in the final mixture was from 0 to 112 mM . The results (Fig. 4) show that the

Fig. 3. Stimulation by sodium in the medium of Q_{O_2} of brain-cortex slices. Slices were incubated in sodium-free medium, choline chloride (●) or sucrose (○) media to which NaCl medium was added to give the sodium concentrations shown on the abscissa. Half-maximal stimulation was produced by 15 mM -sodium in sucrose medium and by 20 mM -sodium in choline chloride medium.

Table 2. *Decrease in Q_{O_2} of brain homogenates in the presence of $5 \mu\text{M}$ -ouabain and with the omission of sodium from the medium*Conditions were as in Fig. 4. Results are given $\pm$ S.E.M.

Soln. in which homogenate made	...	...	...	...	NaCl	KCl	Choline chloride	Sucrose
No. of experiments	...	...	...	...	3	5	4	4
Sodium-rich medium	{ No ouabain (a) { With ouabain (b) { Change due to ouabain (c) = (a) - (b)				2.70 1.40 1.30	2.62 $\pm$ 0.23 1.69 $\pm$ 0.07 0.93 $\pm$ 0.23	2.33 $\pm$ 0.19 1.59 $\pm$ 0.14 0.74 $\pm$ 0.18	1.65 $\pm$ 0.11 1.09 $\pm$ 0.06 0.56 $\pm$ 0.08
Sodium-free medium	{ No ouabain (d) { With ouabain (e) { Change due to ouabain (f) = (d) - (e)				— — —	1.57 $\pm$ 0.14 1.51 $\pm$ 0.11 0.06 $\pm$ 0.10	1.49 $\pm$ 0.10 1.43 $\pm$ 0.07 0.06 $\pm$ 0.09	1.11 $\pm$ 0.10 1.00 $\pm$ 0.06 0.11 $\pm$ 0.05
Fall due to omission of sodium from medium	{ No ouabain (g) = (a) - (d) { With ouabain (h) = (b) - (e)				— —	1.05 $\pm$ 0.32 0.18 $\pm$ 0.13	0.84 $\pm$ 0.20 0.16 $\pm$ 0.10	0.54 $\pm$ 0.08 0.09 $\pm$ 0.06
Ratio: Fall due to omission of sodium (g)					—	1.07 $\pm$ 0.14	1.21 $\pm$ 0.27	1.02 $\pm$ 0.16
Ratio: Fall due to addition of ouabain (c)					—

Fig. 4. Stimulation by sodium in the medium of Q_{O_2} of homogenates of brain cortex. Samples (1.0 ml.) of homogenates which were made in sodium-free medium [either choline chloride (●) or sucrose (○)] were incubated with 3.0 ml. of the same medium or with 3.0 ml. of a mixture of the same medium and NaCl medium which contained the sodium concentrations shown on the abscissa. Half-maximal stimulation was produced by 15 mM-sodium.

addition of about 15 mM-NaCl was sufficient for half-maximal stimulation. The homogenate also contained sodium that was present in the original tissue and analysis showed this to be about 10 mM. The total sodium concentrations in the homogenates were therefore 10 mM higher than the concentrations added in the media; this means that the sodium concentration producing half-maximal stimulation of Q_{O_2} of homogenates was 25 mM. The comparable value with slices is 15 mM.

Half-maximal inhibition by ouabain. The concentration of ouabain that produced half-maximal inhibition of Q_{O_2} was found to be about $1 \mu\text{M}$.

DISCUSSION

Inhibition of active cation transport

Ouabain. The results have shown that the addition of ouabain to a sodium-rich medium or the omission of sodium from the medium cause the

same loss of intracellular tissue potassium, although the mechanism appears to be different. Ouabain inhibits active ion movements in erythrocytes (Schatzmann, 1953; Kahn & Acheson, 1955; Glynn, 1957), and it is thought to be a specific inactivator of active cation transport. It appears to inhibit from outside cells and not from inside (Caldwell & Keynes, 1959), and it is not surprising that small concentrations (about $1 \mu\text{M}$) cause a 70% loss of potassium from brain slices. Brain cortex is therefore one more kind of tissue that loses potassium when exposed to ouabain, the amount lost being that which is otherwise maintained by a mechanism dependent on the presence of sodium in the medium.

Sodium-free media. In the absence of sodium in the medium, very little sodium was found in the tissue and the concentration gradient between intra- and extra-cellular fluid and the active efflux must have been abolished. The failure of the slices to retain potassium in sodium-free choline chloride medium may be explained if the movement of potassium into the cells is linked to, and is a consequence of, the outward movement of sodium [as it is in erythrocytes (Harris & Maizels, 1951; Glynn, 1956), striated muscle (Keynes, 1954; Keynes & Swan, 1959) and nerve axons (Hodgkin, 1958)]. Whatever the detailed nature of the sodium and potassium movements in brain slices, the removal of extracellular sodium indubitably causes a loss of intracellular sodium and potassium, and, moreover, the concentration of potassium is not further decreased by the addition of ouabain. The active transport of potassium was therefore stopped both by ouabain and by the removal of sodium from the incubation medium.

Regulation of respiration by active transport

Biochemical reactions yielding energy are far better understood than those utilizing energy (see Krebs & Kornberg, 1957), and only few attempts

have been made to see how much of the available energy is used by different endergonic processes. Muscle has been studied from this point of view because a comparison can be made between the mechanical work done and the metabolic energy produced. Of other tissues, brain is, perhaps, most interesting because of the close dependence of its function upon respiration, which depends upon a supply of glucose and oxygen from the blood (see McIlwain, 1959). One of the functions of brain is the conduction of stimuli both along and between cells and this is accompanied by electrical potential changes. Electrical stimulation of slices of cortex *in vitro* causes a loss of potassium (McIlwain, 1961), which resembles the loss found during conduction along squid giant axons (Hodgkin, 1958). It therefore seems likely that electrical excitation in brain cortex, as in peripheral nerve axons, leads to a passive outward movement of potassium and probably also to an inward migration of sodium. Since the brain is continually active electrically, the reversal of these ionic shifts by active transport must constantly occur and require energy from metabolism. The present results suggest how much of the normal respiration is required for, and is regulated by, the active transport system which maintains the high tissue-potassium concentration *in vitro*.

In experiments with slices it was found that, when a loss of tissue potassium was induced either by ouabain or by the removal of Na^+ ions from the medium, it was accompanied by the same fall in Q_{O_2} . The parallelism of the effects suggests that the reduction in Q_{O_2} is a consequence of the primary cessation of active cation transport. Thus just as respiration is needed to maintain a supply of energy for active cation transport, so the latter appears to regulate a part of the respiration according to the energy requirement for osmotic work. The active transport system, as a generator of ADP, thus appears partly to control the respiration of the slices. Indeed, about 40 % of the respiration of brain cortex depends upon activation by Na^+ ions and seems to be tightly coupled to active transport, suggesting that respiration and active transport act as pacemakers for each other. Wollenberger (1947) described a similar inhibition by cardiac glycosides of the respiration of guinea-pig-brain slices.

The respiration of homogenates was lowered in the same way as that of slices, suggesting that the mechanism which was sensitive to ouabain and to sodium in the slice remained sensitive after breaking up the cells. This would appear to be the active transport system functioning in the homogenate as an adenosine triphosphatase and thereby stimulating respiration by its generation of ADP. There is, of course, the possibility that other ATP-

hydrolysing mechanisms besides active transport may also be sensitive to ouabain and sodium but this seems somewhat unlikely in view of the adenosine triphosphatases of erythrocytes (Post, Merritt, Kinsolving & Albright, 1960; Dunham & Glynn, 1961), kidney (Whittam & Wheeler, 1961), brain (Hess & Pope, 1957; Järnefelt, 1961; Deul & McIlwain, 1961) and crab nerve (Skou, 1957, 1960), which are inhibited in fragmented cells by compounds that inhibit active transport in the intact cells. The present results extend these observations on the hydrolysis of ATP in showing that the Q_{O_2} is similarly inhibited (by ouabain) and stimulated (by sodium) both in tissue slices and homogenates.

SUMMARY

1. An attempt has been made to find out whether the energy-requiring uptake of K^+ ions acts as a pacemaker for respiration in slices of rabbit-brain cortex. The respiration of both slices and homogenates has been measured under conditions of incubation such that the active transport was inhibited without the use of respiratory poisons.

2. The uptake and the retention of potassium by slices during incubation in a saline medium containing glucose was inhibited by low concentrations of the glycoside ouabain, about $0.5 \mu\text{M}$ being required to produce half-maximal inhibition. With maximum inhibition, the potassium concentration of the tissue was only 30 % of that in the control. Ouabain also caused about a 40 % fall in the Q_{O_2} of slices under conditions when potassium transport was inhibited. The same results were obtained when the medium contained glucose plus glutamate.

3. Incubation of slices in a medium in which sodium had been replaced with choline caused falls in the potassium concentration and in the Q_{O_2} , each of which was not further decreased by the addition of ouabain.

4. The respiration of homogenates was also depressed by the addition of ouabain ($5 \mu\text{M}$) in a high-sodium medium and by the replacement of the medium sodium with potassium, choline or sucrose; under the last-named conditions, as was found with slices, no further fall occurred with ouabain.

5. About 40 % of the respiration and the active uptake of potassium by brain cortex are inhibited by ouabain and by deprivation of the medium of sodium. The inhibition by ouabain of both potassium transport and respiration was manifest only in the presence of sodium in the medium. A cell component connected with the process of active transport and equally sensitive to inhibition by ouabain and to activation by sodium therefore appears to be capable of controlling about 40 % of the tissue's respiration.

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The Isolation of Albumin from Blood Serum or Plasma by means of Organic Solvents

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It has been known for some time that the trichloroacetic acid precipitates of certain proteins dissolve in methanol or ethanol whereas those of other proteins do not. Thus Delaville (1954), having shown that the trichloroacetic precipitate of albumin was soluble in alcohol, but that those of the globulins were alcohol-insoluble, utilized this distinguishing feature for the analysis of serum. He attributed the solubilizing action to some special feature of trichloroacetic acid coupled with its ability to form a very strong bond with albumin.

Several authors (Korner & Debro, 1956; Schwert, 1957; Kallee, Lohss & Oppermann, 1957) have subsequently investigated the properties of albumin after treatment with trichloroacetic acid and alcohol or acetone and have arrived at the

conclusion that the albumin, after recovery, is still in the native state, as judged by its physico-chemical properties and by its unchanged antigenic properties, but that it has acquired a slightly changed susceptibility towards proteolytic enzymes (Ram & Maurer, 1958).

A more detailed study of the behaviour of the main blood proteins and some other proteins has now shown that the solubilizing action in suitable organic solvents, on albumin, but not on globulins, is shared by a number of other acids. Some acids were found to be unable to prevent damage to the albumin in the organic solvents, whereas others preserved the albumin in a condition that is more nearly related to the native state.

This has enabled a process of separation of blood