2. The nuclei *in vitro* incorporate [2-^{14}C]glycine and [1-^{14}C]valine into nuclear protein and [6-^{14}C]-orotic acid and [8-^{14}C]adenine into nucleic acids.

3. Anoxia and 2,4-dinitrophenol inhibit the incorporation of the radioactive precursors into nuclear proteins and nucleic acids. Chlorpromazine inhibits the incorporation of valine, but has no effect on the incorporation of orotic acid.

4. The specific activities of adenosine triphosphatase, succinoxidase, cytochrome oxidase, DPNH–cytochrome *c* reductase, DPNH–neotetrazolium reductase and lactic dehydrogenase are reported. Evidence suggesting that succinoxidase activity is due to mitochondrial contamination whereas cytochrome oxidase is a true nuclear component is presented.

5. The chemical composition and metabolism of rat-kidney nuclei and rat-liver nuclei are compared.

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Adenosine Triphosphatase in the Microsomal Fraction from Rat Brain

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Skou (1957) isolated a microsomal fraction from an homogenate of the leg nerves of the shore crab which contained an adenosine triphosphatase requiring magnesium; to obtain maximum activity both Na^+ and K^+ ions were necessary (Skou, 1957). From the results of detailed studies of the ionic requirements for this adenosine triphosphatase, Skou (1957, 1960) suggested that this enzyme was involved in the active extrusion of sodium from the nerve fibre.

Later work on the hydrolysis of ATP by erythrocyte membranes has also suggested that this process is involved in the maintenance of the ionic composition of the mammalian erythrocyte. The main basis for this conclusion is the quantitative similarity of the ionic requirements for both systems (Mg^{2+}, Na^+ and K^+ ions) and their inhibition by similar concentrations of cardiac glycosides (Post 1959; Post, Merritt, Kinsolving & Albright, 1960; Dunham & Glynn, 1960, 1961; Glynn, 1957*a*).

Adenosine triphosphatase has been found in mammalian nervous tissue: Abood & Gerard (1954) found it in a microsomal fraction from an homogenate of rat nerve, and in disrupted rat brain it is stimulated by magnesium, sodium and potassium (Utter, 1950; Hess & Pope, 1957). Järnefelt (1961) obtained a stimulation of 15–30% by Na^+ ions alone with a microsomal fraction from rat brain. In a preliminary note Deul & McIlwain (1961) showed also that, in the presence of potassium, sodium stimulated (2·5-fold) the adenosine triphosphatase of a microsomal fraction from guinea-pig cerebral cortex.

The present paper describes the isolation of a microsomal fraction from whole rat brain and the properties of the adenosine triphosphatase present in it.

METHODS

Homogenization. In initial experiments the Potter–Elvehjem homogenizer with a Perspex pestle (total difference in diameter between tube and pestle, 0·010 in.; speed of rotation, 1100 rev./min.) was used (Aldridge, Emery & Street, 1960). Although good preparations could be obtained in this way, more reproducible preparations were obtained with a M.S.E. homogenizer (Measuring and Scientific Equipment Ltd.) fitted with a tachometer. With the 10 ml. vortex flask, one rat brain was homogenized in 7·5 ml. of ice-cold 0·3 M-sucrose for 2 min. at 9000 rev./min.

The tube was surrounded by ice and after homogenization the final volume was made up to 12·5 ml. In practice two rat brains were homogenized separately, and the homogenates combined and diluted to 25 ml.

Isolation of microsomal fraction. To speed up the isolation procedure, intact cells, nuclei and mitochondria were removed in a single fast spin in the Spinco Model L centrifuge at 62 000*g* for 5 min. The time, measured with a stopwatch, was taken from the starting of the centrifuge motor to when it was switched off. Since the times for acceleration and deceleration are the same, the total centrifugal force × time is 310 000*g* min. This is equivalent to the total *g* min. used in the procedure described by Aldridge & Johnson (1959) for the separation of the nuclear and mitochondrial fractions of rat brain. The supernatant was carefully poured off, diluted with 0·3 M-sucrose to fill the centrifuge tube, and centrifuged at 145 000*g* for 30 min. The supernatant was discarded, the tube drained and wiped inside with filter paper, and the pellet was suspended in 0·3 M-sucrose. The microsomal fraction from two rat brains was diluted to 6 ml. and stored in an ice bath. All *g* values refer to the bottom of the tubes and all procedures were carried out as near 0° as possible.

Determination of adenosine-triphosphatase activity. The solution in each tube contained $MgCl_2$ (2 mM), EDTA (tris salt) (1 mM, approx. pH 7·4), tris–tris chloride (40 mM, pH 7·4). To give almost maximum stimulation NaCl (150 mM) and KCl (15 mM) were also added. The microsomal fraction (0·1 ml.) was added to the ice-cold medium in the tubes, followed by the ATP (tris salt) (5 mM, approx. pH 7·4). The concentrations given are those in the total volume of 2 ml. The reaction was started by placing the tubes in a bath at 37°; incubation was continued usually for 10 min. and then stopped by the addition of 3 ml. of ice-cold 0·85 N-perchloric acid to each tube. The tubes were placed in an ice bath and then centrifuged at 0°. Inorganic phosphate was determined by a modification of the method of Fiske & Subbarow (1925). The samples (4 ml.) was added to 25 ml. flasks (in an ice bath) each containing the sulphuric acid, ammonium molybdate and water to about 15 ml. The reducing agent was then added, followed by dilution to 25 ml. The colour was developed by placing the flasks in a bath of running tap water. This whole procedure ensured that the blank due to acid-catalysed hydrolysis of ATP was low (not more than 0·25 μg.atom of P/tube) and consistent throughout the samples. (This was because the rate of hydrolysis of ATP in the acid molybdate was negligible at 0° and was consistent in all tubes during colour development.) The results are expressed as μg.atoms of P liberated as inorganic phosphate/mg. of protein/hr. The liberation of inorganic phosphate under these conditions was linear with time up to 10 min., and to enzyme concentration provided that not more than 2·5 μg.atoms of P were liberated.

Special chemicals. ATP (disodium salt), from Sigma Chemical Co., St Louis, Mo., U.S.A., was converted into the free acid by passing it through a column of Dowex 50 (50–100 mesh, H^+ form) and neutralizing it to approx. pH 7·4 with tris. This procedure reduced the concentration of sodium in the original stock 0·05 M-ATP from 0·1 M to 0·08 mM. The concentration of sodium, added in the ATP, did not therefore exceed 8 μM in the assay medium. This procedure is more efficient than passing the ATP through a column of Dowex 50 (tris form).

Analytical methods. Protein was determined by the biuret method of Robinson & Hogden (1940), as modified by Aldridge (1957). The protein was precipitated by perchloric acid at a final concentration of 0·63 N and the suspensions were stored at 5°. After centrifuging, the pellet of protein was suspended in ethanol–ether (1:1, v/v). The suspension was brought to approx. pH 7 (Universal indicator, used internally) by the addition of drops of 2% (w/v) NaOH. The volume was finally adjusted to approx. 10 ml. with ethanol–ether (1:1); the solution was left for at least 10 min. at room temperature, the protein was centrifuged down and the tube inverted and drained. This procedure prevents cloudiness (presumably due to lipid) during development of the final biuret colour.

RESULTS

Methods of isolation of the microsomal fraction

Three criteria have been used to assess the value of methods of homogenization, of isolation and of assay of adenosine-triphosphatase activity: (*a*) the specific activity of the enzyme; (*b*) the degree of stimulation on the addition of sodium and potassium; (*c*) the reproducibility of the preparation. A high specific activity and a high degree of stimulation have been regarded as the properties of a good preparation.

When a bladed homogenizer was used instead of the Potter–Elvehjem type found suitable for the preparation of intact mitochondria (Aldridge *et al.* 1960; Aldridge & Johnson, 1959), a more consistent preparation was obtained with a high specific activity and showing a 2·5-fold stimulation.

When brain slices are stored at 0°, basic histones migrate from the nuclei to the microsomal fraction containing the gangliosides and the slices lose their ability to be stimulated by electrical pulses (Wolfe & McIlwain, 1961; Wolfe, 1961). It was considered possible that such a migration might occur in the homogenate during the isolation procedure; therefore, instead of a separate removal of the nuclei and

Table 1. *Effect of* pH *on the activity of the adenosine triphosphatase of the microsomal fraction from rat brain*

The tris buffer was adjusted to the required pH by hydrochloric acid with a glass electrode. For the stimulated activity sodium chloride (150 mM) and potassium chloride (15 mM) were added.

	Activity (μg.atoms of P/mg. of protein/hr.)		
pH	Without Na^+ or K^+ ions (*a*)	With Na^+ plus K^+ ions (*b*)	Stimulation (*b*/*a*)
6·8	14·0	21·8	1·6
7·1	13·2	22·3	1·7
7·4	12·8	25·9	2·0
7·7	11·8	25·6	2·2

Table 2. *Effect of magnesium and ethylenediaminetetra-acetic acid on the activity of the adenosine triphosphatase of the mitochondrial fraction from rat brain*

For the stimulated activity sodium chloride (150 mM) and potassium chloride (15 mM) were added.

EDTA present or absent	Concn. of Mg^{2+} ions (mM)	Activity (μg.atoms of P/mg. of protein/hr.) Without Na^+ or K^+ ions (a)	With Na^+ plus K^+ ions (b)	Stimulation (b/a)
–	1	10·5	22·4	2·1
+	1	6·3	13·0	2·1
+	2	10·5	25·3	2·4
+	3	14·2	32·1	2·3
+	4	18·6	36·2	1·9
+	5	21·6	38·4	1·8

mitochondrial fractions, the time of contact between nuclei and microsomes was reduced and the nuclei and mitochondria were removed by one short fast spin. This improved neither the specific activity nor the degree of stimulation by Na^+ plus K^+ ions.

In several experiments the microsomal fraction was washed once with 0·3 M-sucrose. This caused a loss of 60–70 % of the protein and 30–40 % of the adenosine-triphosphatase activity. Thus the specific activity increased but the degree of stimulation obtained by sodium and potassium remained the same (Table 4). Although an increased specific activity could be obtained, washing was not usually carried out because of the increased time for the preparation and the loss of adenosine-triphosphatase activity.

Properties of the adenosine-triphosphatase activity

Effect of pH. The processes involved were not very sensitive to changes of pH. The degree of stimulation obtained on the addition of sodium plus potassium increased slightly as the pH increased from 6·8 to 7·4, but the change was marginal and near to the accuracy of the method. Both tris and triethanolamine buffers appeared to be suitable: tris was used because it is easier to purify by recrystallization.

Effect of EDTA. The addition of EDTA (1 mM) to the medium produced no change in the degree of stimulation by sodium plus potassium but lowered proportionately the activity both with and without sodium plus potassium. This was presumably due to chelation of magnesium but the fact that some activity was obtained in the presence of magnesium (1 mM) and EDTA (1 mM) was an indication of the high affinity of the enzyme for magnesium. The addition of EDTA (1 mM) in the absence of added magnesium prevented enzyme activity. The stimulation obtained on addition of sodium plus potassium was slightly higher in the presence of EDTA (magnesium, 2 mM; EDTA, 1 mM) than its equivalent without EDTA (magnesium, 1 mM; no EDTA). For this reason and as a safeguard against contamination of the preparation or medium with other metals, EDTA was used in the assay medium. No advantage was found by adding EDTA (1 mM) to the 0·3 M-sucrose used during isolation of the microsomal fraction.

Effect of magnesium. Increases in the concentration of magnesium stimulated the activity both with and without sodium plus potassium (Table 2). Maximum stimulation was obtained with 2 mM-magnesium plus 1 mM-EDTA.

Effect of calcium. In experiments carried out without EDTA in the medium, calcium stimulated the adenosine triphosphatase in the absence of sodium and potassium but inhibited it in their presence (Table 3). In the presence of EDTA, calcium (0·5 mM and lower) stimulated the activity in the absence of sodium plus potassium, but also stimulated the activity by the same amount in their presence. The inhibitory activity of calcium in the presence of sodium and potassium was therefore prevented by EDTA, but its stimulatory action in the absence of sodium and potassium was virtually unaffected. At a concentration of calcium which was higher than that of free Mg^{2+} ions, calcium inhibited the activity in the presence of sodium plus potassium. Although the interpretation of these experiments is difficult, the addition of EDTA prevents the fall in the degree of stimulation produced by concentrations of calcium less than 0·5 mM.

Effect of fluoride. Sodium fluoride produced a slight stimulation of adenosine triphosphatase in the absence of sodium plus potassium, and inhibition at the higher concentrations (3 mM and above). The high specific activity of the enzyme in this experiment is due to the use of a washed preparation (see above). This result is of some interest as deleterious effects due to contamination of brain mitochondria by this enzyme are unlikely to be prevented by 10 mM-sodium fluoride.

Table 3. *Effect of calcium on the activity of the adenosine triphosphatase of the microsomal fraction from rat brain*

For the stimulated activity sodium chloride (150 mM) and potassium chloride (15 mM) were added. In Expt. 1, the medium contained Mg^{2+} ions (1·0 mM) but no EDTA; in Expt. 2, the medium contained both Mg^{2+} ions (2·0 mM) and EDTA (1·0 mM).

No. of expt.	Concn. of Ca^{2+} ions (mM)	Activity (μg.atoms of P/mg. of protein/hr.) Without Na^+ or K^+ ions (*a*)	With Na^+ plus K^+ ions (*b*)	Stimulation (*b*/*a*)
1	0	11·1	22·9	2·1
	0·004	11·1	23·7	2·1
	0·02	12·4	22·5	1·8
	0·10	13·9	21·5	1·5
	0·50	14·6	19·5	1·3
	2·5	17·8	19·3	1·1
2	0	10·8	25·6	2·4
	0·004	10·1	26·9	2·7
	0·02	10·1	26·2	2·6
	0·10	11·6	28·7	2·5
	0·50	14·7	30·8	2·1
	2·5	17·1	20·6	1·2

Table 4. *Effect of sodium fluoride on the activity of the adenosine triphosphatase of the microsomal fraction from rat brain*

The preparation used was washed once with 0·3M-sucrose. For the stimulated activity sodium chloride (150 mM) and potassium chloride (15 mM) were added.

Concn. of NaF (mM)	Activity (μg.atoms of P/mg. of protein/hr.) Without Na^+ or K^+ ions (*a*)	With Na^+ plus K^+ ions (*b*)	Stimulation (*b*/*a*)
0	19·6	50·9	2·6
0·12	21·8	52·3	2·4
0·37	23·3	52·3	2·2
1·11	25·1	50·9	2·0
3·33	25·1	46·1	1·8
10·0	21·1	29·4	1·4

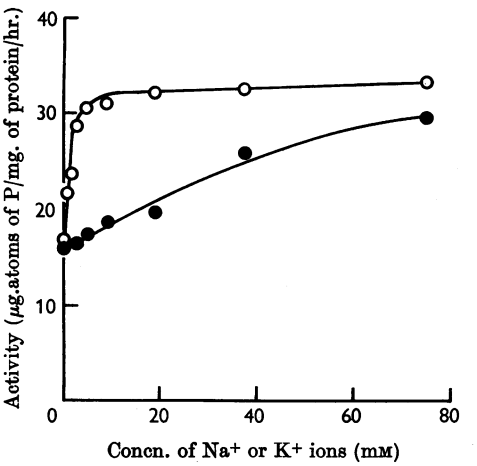

Fig. 1. Effect of Na^+ and K^+ ions on the activity of the adenosine triphosphatase of the microsomal fraction from rat brain; ●, sodium chloride in the presence of potassium chloride (150 mM); ○, potassium chloride in the presence of sodium chloride (150 mM). In the absence of sodium chloride and potassium chloride the activity was 12·8, and in the presence of sodium chloride (150 mM) plus potassium chloride (150 mM) it was 33·8 μg.atoms of P/mg. of protein/hr.

Stimulation of enzyme activity by sodium and potassium

Maximum adenosine-triphosphatase activity depended on the addition of both sodium and potassium, neither of which produced an appreciable stimulation when added separately. For instance potassium chloride (150 mM) stimulated activity 1·2-fold and sodium chloride (150 mM) 1·3-fold (Fig. 1). However, if the medium contained sodium chloride (150 mM), the addition of potassium chloride caused a large increase in activity (2·0 times that with sodium chloride alone and 2·7 times that without sodium chloride). The concentration of potassium producing half-maximal stimulation was approx. 1·0 mM; 20 mM produced almost maximum activity. In the presence of a high potassium concentration (150 mM) the activity increased less relative to the increasing sodium concentrations and over 100 mM was required for maximum activity. The concentration of sodium causing half maximum activation was approx. 30 mM.

The requirement for both sodium and potassium eliminated the possibility that the stimulation is a simple ionic effect or that it is due to chloride. Controls containing sucrose to correct for the difference in osmolarity between those with and those without sodium and potassium were identical with those not containing sucrose.

Table 5. *Effect of lithium, rubidium and caesium on the activity of the adenosine triphosphatase of the microsomal fraction from rat brain*

Each ion was added as the chloride.

No. of expt.	Additions	Activity (μg.atoms of P/mg. of protein/hr.)
1	None	11·0
	Na^+ (150 mM) + K^+ (15 mM)	25·8
	Li^+ (150 mM)	14·1
	Li^+ (150 mM) + K^+ (0·6–75 mM)	13·0–15·1
	Na^+ (150 mM)	12·5
	Na^+ (150 mM) + Li^+ (1·2 mM)	12·8
	Na^+ (150 mM) + Li^+ (4·7 mM)	14·1
	Na^+ (150 mM) + Li^+ (18·7 mM)	15·7
	Na^+ (150 mM) + Li^+ (75 mM)	20·2
2	None	10·9
	Na^+ (150 mM) + K^+ (15 mM)	26·6
	Rb^+ (150 mM)	12·8
	Rb^+ (150 mM) + K^+ (0·6–150 mM)	11·3–12·8
	Na^+ (150 mM)	14·7
	Na^+ (150 mM) + Rb^+ (0·6 mM)	18·0
	Na^+ (150 mM) + Rb^+ (1·2 mM)	20·2
	Na^+ (150 mM) + Rb^+ (2·3 mM)	20·9
	Na^+ (150 mM) + Rb^+ (9·3 mM)	23·4
	Na^+ (150 mM) + Rb^+ (75 mM)	25·3
3	None	11·9
	Na^+ (150 mM) + K^+ (15 mM)	29·9
	Cs^+ (150 mM)	13·6
	Cs^+ (150 mM) + K^+ (0·6–150 mM)	12·5–14·1
	Na^+ (150 mM)	17·7
	Na^+ (150 mM) + Cs^+ (1·2 mM)	17·3
	Na^+ (150 mM) + Cs^+ (4·7 mM)	21·4
	Na^+ (150 mM) + Cs^+ (18·7 mM)	25·9
	Na^+ (150 mM) + Cs^+ (75 mM)	28·5

Table 6. *Effect of ammonium and choline on the activity of the adenosine triphosphatase of the microsomal fraction from rat brain*

Each ion was added as the chloride.

No. of expt.	Additions	Activity (μg.atoms of P/mg. of protein/hr.)
1	None	11·8
	Na^+ (150 mM) + K^+ (15 mM)	28·2
	NH_4^+ (150 mM)	12·7
	NH_4^+ (150 mM) + K^+ (0·6–150 mM)	10·9–12·7
	Na^+ (150 mM)	16·2
	Na^+ (150 mM) + NH_4^+ (0·6 mM)	16·4
	Na^+ (150 mM) + NH_4^+ (1·2 mM)	18·8
	Na^+ (150 mM) + NH_4^+ (2·3 mM)	19·4
	Na^+ (150 mM) + NH_4^+ (4·7 mM)	22·4
	Na^+ (150 mM) + NH_4^+ (9·3 mM)	24·5
	Na^+ (150 mM) + NH_4^+ (37·5 mM)	29·8
2	None	11·7
	Na^+ (150 mM) + K^+ (15 mM)	27·6
	Choline (150 mM)	11·0
	Choline (150 mM) + K^+ (6–150 mM)	11·0
	Na^+ (150 mM)	15·9
	Na^+ (150 mM) + choline (6 mM)	15·9
	Na^+ (150 mM) + choline (30 mM)	14·4
	Na^+ (150 mM) + choline (150 mM)	12·4

Table 7. *Effect of ouabain on the activity of the adenosine triphosphatase of the microsomal fraction from rat brain*

The stimulated activity was determined in the presence of sodium chloride (150 mM) and potassium chloride (15 mM). The microsomes were incubated with ouabain in the medium without ATP for 10 min. at 0°.

Concn. of ouabain (μM)	Activity (μg.atoms of P/mg. of protein/hr.) Without Na^+ or K^+ ions	With Na^+ plus K^+ ions
0	12·6	30·6
0·01	12·6	30·6
0·1	13·1	30·1
1·0	13·1	25·9
10	13·1	21·3
100	13·1	18·4

The slight stimulation obtained on addition of sodium or potassium alone may be due to the presence of both in the microsomal fraction. Washing the fraction did not lower it, but passing the suspensions of microsomes through a Dowex 50 column (tris form) lowered the stimulation obtained by sodium alone and completely prevented that by potassium alone, though not affecting the degree of stimulation obtained on the addition of both.

Effect of other univalent ions

The effects of lithium, rubidium and caesium have been studied in a series of experiments in which these replaced either sodium or potassium (Table 5). Lithium, rubidium or caesium did not individually stimulate adenosine-triphosphatase activity, and therefore resembled sodium and potassium in this respect. Replacement of sodium by lithium, rubidium or caesium completely eliminated the stimulatory properties of potassium shown in the presence of sodium. However, all of these could replace potassium and the enzymic activity in the presence of sodium is increased by lithium, rubidium and caesium. The maximal activity obtained with rubidium and caesium was the same as that with potassium, but that with lithium was considerably less. Ammonium ions behaved like rubidium and caesium and could replace potassium completely (Table 6). Choline, on the other hand, could not replace sodium or potassium and appeared to be completely inert in this system. In this respect it resembles tris buffer.

Effect of ouabain

Since the cardiac glycoside, ouabain (Strophanthin G), inhibits the active transport of cations in a variety of tissues, the influence of ouabain on the sodium-plus-potassium-stimulated adenosine triphosphatase was examined (Table 7). Ouabain up to a concentration of 100 μM did not influence the

adenosine-triphosphatase activity in the absence of sodium and potassium. Some inhibition of the sodium-plus-potassium-stimulated activity was obtained between 0·1 and 1·0 μM-ouabain, and 50% inhibition was produced by approx. 6 μM-ouabain, but complete inhibition was not obtained even by 100 μM-ouabain. The inhibition was not increased by preincubation for 10 or 30 min. at 0° or 37°.

DISCUSSION

An adenosine triphosphatase requiring both sodium and potassium for maximum activity has been demonstrated in erythrocyte membranes and crab nerve. In a preliminary finding Deul & McIlwain (1961) reported its presence in guinea-pig cerebral cortex. In this paper the hydrolysis of ATP by a microsomal fraction from rat brain is also shown to require both sodium and potassium for maximal activity. As with the preparation of Deul & McIlwain (1961), the activity obtained in the presence of sodium and potassium is 2·5 times that in their absence. A similar stimulation was obtained by Post *et al.* (1960) and Dunham & Glynn (1961) for erythrocyte membranes, whereas Skou (1960) obtained a sevenfold stimulation. No explanation can be given for the failure of Järnefelt (1961) to demonstrate a requirement for potassium.

In the absence of sodium or potassium, 40% of the maximum activity is obtained. It is difficult to decide whether this is an entirely different process, but its insensitivity to ouabain (Table 7) and fluoride (Table 4) suggests that it is.

Much circumstantial evidence supports the view that the sodium-plus-potassium-stimulated adenosine triphosphatase found in erythrocyte membranes is a part of the processes involved in ion transport in erythrocytes. Whether the adenosine triphosphatase described in this paper is involved in ion transport in brain is not certain, though there is as yet nothing specifically against this view. The enzyme is present in a fraction which has been shown to contain membranous material (Hanzon & Toschi, 1959; Toschi, 1959; Frontali & Toschi, 1958) and the enzyme appears to be specific (so far as the present studies are concerned) for sodium. Neither alkali metal nor ammonium can replace sodium, but they can all replace potassium in activating the enzyme in the presence of sodium. Nevertheless, the enzyme requires for maximum activity both the physiological ions sodium and potassium, and this may be correlated with the view that sodium transport depends on the presence of potassium in erythrocytes (Glynn, 1957*b*) and in giant nerve axons of squids and cuttle fish (Hodgkin & Keynes, 1955). Perhaps more pertinent is the observation that ouabain inhibits the adenosine triphosphatase stimulated by sodium plus potassium. Evidence is now accumulating that ouabain inhibits cation transport in a variety of tissues: in intact erythrocytes (Schatzmann, 1953; Kahn & Acheson, 1955), in brain slices (Yoshida, Nukada & Fujisawa, 1961), in calf lens (Kinoshita, Kern & Merola, 1961) and in kidney slices (Whittam, 1961). Ouabain also inhibits the adenosine triphosphatase stimulated by sodium plus potassium obtained from a variety of sources: from erythrocytes (Dunham & Glynn, 1961; Post *et al.* 1960), from kidney (Whittam & Wheeler, 1961) and from crab nerve (Skou, 1960). Although there is reasonable agreement between the concentration of ouabain inhibiting cation transport in the erythrocyte (Solomon, Gill & Gold, 1956; Gill & Solomon, 1959; Kahn & Acheson, 1955) and that inhibiting the adenosine triphosphatase stimulated by sodium plus potassium (Post *et al.* 1960; Dunham & Glynn, 1961), not enough quantitative information is available to attempt the correlation for other tissues. The interpretation of the inhibiting action of ouabain on the adenosine triphosphatase must await further work establishing the specificity of ouabain as an inhibitor of cation transport.

Potassium stimulates adenosine-triphosphatase activity in the presence of sodium. Rubidium, caesium and ammonium can reproduce exactly this action of potassium and, although maximum activity is not obtained, lithium also has a potassium-like activity. These results agree with those obtained by Skou (1960) on a microsomal fraction from crab nerve. Potassium also stimulates an increased respiration and aerobic glycolysis in brain slices (Ashford & Dixon, 1935; Dickens & Greville, 1935; Dixon, 1937) and this stimulation depends on the presence of sodium (Dickens & Greville, 1935). A similar effect is produced by rubidium and caesium (Dixon & Holmes, 1935; Dickens & Greville, 1935) and ammonium (Weil-Malherbe, 1938). It is tempting to suggest that the stimulated respiration of brain slices is due to stimulation of adenosine triphosphatase in the membranous structures of the slices. In this context the similar sensitivity to barbiturates of the potassium-stimulated and the electrically stimulated respiration in brain slices is interesting (McIlwain, 1953). However, these systems are not always equally sensitive to drugs, for atropine inhibits respiration stimulated by electrical pulses but not that stimulated by potassium (McIlwain, 1951).

SUMMARY

1. The isolation is described of a microsomal fraction from rat brain possessing an adenosine triphosphatase which is stimulated 2·5-fold by sodium plus potassium. The presence of both ions is required for maximal activity.

2. The effect of varying the conditions of isolation of the microsomal fraction and the determination of enzymic activity has been studied.

3. The influence of other univalent ions (Li^+, Rb^+, Cs^+, NH_4^+ and choline) has been determined.

4. The possible connexion between this adenosine triphosphatase and sodium transport is discussed.

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Carbohydrate Substrates for Skin Cells

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Thin slices of mammalian skin can be maintained *in vitro* on an appropriate buffer solution to which various sugars, e.g. glucose or fructose, have been added. Respiration proceeds at a steady rate and the sugars are utilized with the formation of lactic acid and carbon dioxide (Cruickshank & Trotter, 1956). When [^{32}P]orthophosphate is added to the medium the phosphorylated intermediates of glycolysis become labelled. After separation by two-dimensional paper chromatography these phosphate esters, though present in amounts too small for detection chemically, are visible on a radioautograph of the chromatogram. In previous work these methods were applied in a study of skin maintained on glucose and the effects of various deoxyglucoses were examined; the inhibition of respiration by 2-deoxyglucose was measured and the formation of 2-deoxyglucose 6-phosphate by skin cells was observed (Brooks, Lawrence & Ricketts, 1959). The present work considerably extends the range of carbohydrates tested as substrates. The effects of 4-, 5- and 6-deoxyglucose and 2-deoxygalactose have been studied.

MATERIALS

Sugars were of the D-series except where stated and all were laboratory reagents except for 4- and 5-deoxyglucose [recently synthesized by Hedgerly & Overend (1960)], and 6-deoxyglucose [synthesized by Dr Sheila Brooks by the