Transport and Metabolism of Acetate in Rat Brain Cortex *in vitro*

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1. [1-^{14}C]Acetate undergoes metabolism when incubated aerobically at 37° in the presence of rat brain-cortex slices, forming $^{14}CO_2$ and ^{14}C-labelled amino acids (glutamate, glutamine, aspartate and relatively small quantities of γ-aminobutyrate). In the absence of glucose the yield of ^{14}C-labelled aspartate exceeds that of ^{14}C-labelled glutamate and glutamine. The addition of glucose brings about a doubling of the rate of formation of $^{14}CO_2$ and a greatly increased yield of ^{14}C-labelled glutamate or glutamine, whereas that of ^{14}C-labelled aspartate is diminished. 2. The addition of potassium chloride (100mM) to the incubation medium causes an increased rate of $^{14}CO_2$ formation in the presence or absence of glucose and an increased rate of utilization of acetate. 3. The addition of 2,4-dinitrophenol (0·1mM) suppresses the rate of utilization of [1-^{14}C]acetate. 4. The presence of ouabain (10μM) suppresses the rate of formation of $^{14}CO_2$ from [1-^{14}C]acetate and the rate of acetate utilization. Acetate conversion into carbon dioxide in the rat brain cortex is both Na^+- and K^+-dependent and controlled by operation of the active sodium-transport process. Only the Na^+-stimulated rate is suppressed by ouabain. 5. Sodium fluoroacetate (1mM) decreases the rate of $^{14}CO_2$ evolution from [1-^{14}C]acetate in the presence of rat brain cortex without affecting the respiratory rate. The results are consistent with the conclusion that fluoroacetate competes with, or blocks, a transport carrier for acetate, so that in its presence only the passive diffusion rate of acetate takes place. 6. The presence of sodium propionate or sodium butyrate suppresses the utilization of [1-^{14}C]acetate in rat brain cortex and leads to a concentration ratio (tissue/medium) of [1-^{14}C]-acetate greater than unity. 7. The presence of NH_4^+ diminishes acetate utilization, this being attributed to a diminished ATP concentration. Glycine is also inhibitory. It is concluded that acetate transport into the brain is carrier-mediated and dependent on the operation of the sodium pump.

Investigations of the metabolism of [1-^{14}C]-acetate in rat brain-cortex slices incubated in a physiological Ringer medium have led us to the conclusion that acetate transport into the brain is carrier-mediated and dependent on the operation of the active sodium-transport process. The results leading to this conclusion, as well as those concerned with the conversion of [1-^{14}C]acetate into ^{14}C-labelled amino acids and the effects of various agents on acetate metabolism, are described in this paper. Preliminary statements on acetate metabolism and transport in the brain *in vitro* have been published (Gonda & Quastel, 1963, 1964; see also Beloff-Chain *et al.* 1962).

MATERIALS AND METHODS

Materials

Sodium [1-^{14}C]acetate (10·0mc/m-mole) was purchased from The Radiochemical Centre, Amersham, Bucks. It was diluted in water and appropriate quantities were added to the incubation vessels. It was stored frozen. Sodium [^{32}P]phosphate solution (1mc/ml.) was purchased from C. E. Frosst and Co., Montreal, Canada, and after dilution it was stored frozen. [U-^{14}C]Glucose was purchased from Merck and Co., Montreal, Canada. Ouabain was supplied by the Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A., and Dowex 50W (X4) resin was obtained from Dow Chemical Co., Montreal, Canada. All other chemicals used were of reagent grade and were used without further purification.

Preparation of brain-cortex slices

Adult hooded rats weighing 100–150g. were killed by stunning and the brains were removed and placed in cold Krebs–Ringer phosphate medium. Brain-cortex slices were prepared with a Stadie–Riggs microtome. The slices (usual total wet wt. 60–70mg./flask) were weighed at once on a torsion balance and then suspended in appropriate media in Warburg manometric vessels chilled with crushed ice. The slices prepared in this manner had a dry weight approx. 16% of the wet weight.

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Incubation procedure

The brain-cortex slices were incubated in a Krebs–Ringer phosphate medium of the following composition: NaCl, 128mM; KCl, 5mM; $CaCl_2$, 3·6mM; $MgSO_4$, 1·3mM; Na_2HPO_4, 10mM (brought to pH 7·4 with N-HCl). The final volume of medium in each Warburg manometric flask was 1ml.

Incubations were carried out in the standard Warburg manometric apparatus. The main compartment of the manometric vessel contained all the constituents of the incubation medium. The CO_2 was absorbed by 20% (w/v) KOH absorbed on rolls of filter paper in the centre wells. The vessels were gassed with O_2 and incubations were carried out at 37°. Except where otherwise indicated, in the Tables below, each vessel contained [1-^{14}C]acetate (1mM). The Q_{O_2} values (μl. of O_2 uptake/mg. dry wt. of tissue/hr.) were calculated at the end of each experiment.

Estimations of radioactivities

Acetate and non-acid-volatile components. After aerobic incubation at 37° for 1hr. the slices were removed, rinsed with a small quantity of Krebs–Ringer phosphate medium (or with medium of similar composition to that used for the incubation), suspended in 5ml. of 80% (v/v) ethanol and homogenized in a Potter–Elvehjem homogenizer. The homogenizer was washed with small portions of 80% ethanol and the washings were added to the original homogenate. The homogenate was kept at 5° for 2hr. and then centrifuged. The ethanolic extract was collected, and the insoluble residue was washed with 1·5ml. of 80% ethanol.

Samples from the ethanolic extract were transferred quantitatively to stainless-steel planchets and 0·1ml. of N-HCl was added to each planchet, which was then dried under an infrared lamp. The radioactivity on the planchet was measured with a Tracerlab counter. This gave a measure of the radioactivities of the non-acid-volatile components (e.g. amino acid present). Control experiments showed that this procedure effectively removes all acid-volatile compounds (i.e. all remaining radioactive acetate) present. Other samples were transferred to stainless-steel planchets and dried after the addition of 0·1ml. of 0·1N-NaOH. The radioactivity found after this treatment gave a measure of the total radioactivity present (i.e. acetate plus non-acid-volatile components present). The difference between the counts of radioactivity found after the NaOH and HCl treatments of the samples gave a measure of the radioactivity of the acetate present. The radioactivity of the non-acid-volatile components was estimated by difference between total radioactivity and the radioactivity of the remaining acetate.

Amino acids. The pooled ethanolic extract was evaporated to dryness at room temperature in a current of air, and the dried extract was dissolved in a small amount of 80% ethanol and spotted by means of a micropipette on Whatman no. 1 filter-paper sheets for two-dimensional chromatography. The solvents used for chromatography were: phase I [butan-2-ol–90% (v/v) formic acid–water (70:11:17, by vol.)]; phase II [90% (w/v) phenol–water–aq. NH_3 (sp.gr. 0·88) (960:110:3, by vol.)].

Amino acids present in the incubation medium were also estimated. For this purpose the sugars and salts were separated from the amino acids as follows. After removal of the slices, the medium was passed through a column (4cm. × 0·8cm.) of Dowex 50W (X4) resin (200–400 mesh). After the column was washed with water, the adsorbed amino acids were eluted from the column with 50ml. of 2N-NH_3 in ethanol and the eluate was evaporated at room temperature in a current of air. The residues were dissolved in a small amount of 80% ethanol and chromatographed two-dimensionally on Whatman no. 1 filter paper as described above. The values for the amino acids obtained by column chromatography were corrected for the loss of amino acids during the procedure. For control purposes, ^{14}C-labelled amino acids were used.

After two-dimensional chromatography the paper was thoroughly dried and placed in contact with Kodak No-Screen X-ray film for 3 days. The films were then developed and the radioactive spots were located on the paper. The radioactivities of the spots on the paper were quantitatively measured by using a Tracerlab counter with a mica window 28mm. in diameter and a thickness of 1·5–1·8mg./cm.^{2}. The spots were divided into sizes to suit the diameter of the counter window. Activities were corrected for the background. The incorporation of ^{14}C into amino acids was calculated as mμg.atoms of ^{14}C derived from [1-^{14}C]-acetate incorporated/100mg. wet wt. of tissue (for details of calculations, see Gonda & Quastel, 1962*a*,*b*).

The radioactivity of the individual amino acids on the filter paper was corrected by a factor due to the decrease in the counting efficiency in the paper. The corrected value of counts/min. of amino acids was transformed to mμg.atoms of ^{14}C derived from [1-^{14}C]acetate by dividing the value by the specific activity of acetate (counts/min./mμmole). Finally, for amino acids found in the tissue, the term expressing mμg.atoms of ^{14}C was corrected for the weight of tissue and expressed/100mg. wet wt. of tissue.

Incorporation of ^{32}P into the nucleotide pyrophosphate fraction

Measurement of nucleotide pyrophosphates. The incorporation of [^{32}P]phosphate into the total nucleotide pyrophosphate (ATP and ADP) was examined in a manner previously described (Ellis & Scholefield, 1962*a*,*b*; Gonda & Quastel, 1962*b*) with a slight modification. After incubation for 1hr. at 37°, the slices were removed from the medium, rinsed with cold Krebs–Ringer phosphate medium and homogenized in the presence of 10ml. of 5% (w/v) trichloroacetic acid in a Potter–Elvehjem homogenizer. After removal of proteins an aqueous suspension of acid-washed charcoal (Norit A) was added (equivalent to approx. 40mg. dry wt.) to the supernatant. The charcoal was sedimented by centrifuging, washed once with 10ml. of 5% (w/v) trichloroacetic acid and twice with 10ml. of water. After the second washing the tubes were drained by inversion and the insides dried with paper tissues. The charcoal residue was resuspended in 2ml. of N-HCl and the suspension was placed in a boiling-water bath for 7min. After cooling, 2ml. of water was added and the suspension was centrifuged.

The radioactivities in 0·2ml. samples were determined with a Tracerlab counter; 1ml. samples were used for the examination of inorganic phosphate. The inorganic phosphate was determined by the method described by

Bartlett (1959). To a 1 ml. sample 2·3 ml. of water, 0·15 ml. of 20 N-H_2SO_4, 0·15 ml. of 5% (w/v) ammonium molybdate and 0·15 ml. of Fiske–Subbarow reagent were added and mixed thoroughly. The solution was heated for 10 min. in a boiling-water bath and the colour produced was read at 830 mμ with a Beckman model B spectrophotometer. In all experiments blank values of the charcoal and trichloroacetic acid solution were obtained. A standard containing 0·1 μM-phosphate was obtained simultaneously. Before the analysis, the solution containing ^{32}P and the charcoal was purified. This was carried out by boiling the solution containing ^{32}P with N-HCl (to decompose the acid-labile phosphorus compounds present). The acid solution containing ^{32}P was then neutralized with N-NaOH. The charcoal was purified by boiling with N-HCl for 1 hr. The acid-washed charcoal was resuspended in pyridine, washed again with water and dried in an oven overnight at 100°.

RESULTS

Formation of $^{14}CO_2$ and ^{14}C-labelled amino acids from [1-^{14}C]acetate in rat brain-cortex slices. Although the addition of sodium acetate (1–10 mM) to a physiological Ringer medium, with either glucose present or glucose absent, brings about no significant change in the rate of oxygen consumption, the use of [1-^{14}C]acetate makes it clear that this substance undergoes metabolic changes in rat brain cortex incubated aerobically *in vitro*. The results shown in Table 1 indicate that [1-^{14}C]-acetate gives rise to $^{14}CO_2$ and ^{14}C-labelled amino acids, namely glutamate, glutamine, aspartate and traces of γ-aminobutyrate; ^{14}C-labelled alanine is formed in too small a quantity to be measured with any accuracy. Under the given experimental conditions 1000 mμmoles of [1-^{14}C]acetate (in the absence of glucose) give rise in 1 hr. to approx. 50 mμmoles of $^{14}CO_2$ and 56 mμmoles of combined ^{14}C-labelled amino acids. Ten times this quantity of [1-^{14}C]acetate yields under the same conditions 100 mμmoles of $^{14}CO_2$ and 135 mμmoles of combined ^{14}C-labelled amino acids. The addition of unlabelled glucose (5 mM) with [1-^{14}C]acetate (1 mM) gives rise to the following changes: (*a*) the yield of $^{14}CO_2$ is doubled; (*b*) the yield of combined ^{14}C-labelled amino acids is increased from 56 mμmoles to 189 mμmoles; (*c*) whereas in the absence of glucose the yield of ^{14}C-labelled aspartate from [1-^{14}C]acetate exceeds that of ^{14}C-labelled glutamate or glutamine, in the presence of glucose the yields of ^{14}C-labelled glutamine and glutamate are considerably increased and that of ^{14}C-labelled aspartate is decreased. A similar phenomenon occurs during the metabolism of [1,2-^{14}C]succinate in the presence of rat brain-cortex slices (Gonda & Quastel, 1962*a*).

The values of yields of ^{14}C-labelled amino acids from [1-^{14}C]acetate given in Table 1 are approximately the total yields, as estimates of their amounts in the incubation medium show that very little leakage of the amino acids from tissue to medium takes place under the given experimental conditions (Tables 2 and 3).

It is noteworthy (Table 1) that the preponderant

Table 1. *Formation of ^{14}C-labelled amino acids and $^{14}CO_2$ from [1-^{14}C]acetate in the presence of rat brain-cortex slices*

Rat brain-cortex slices from adult hooded rats weighing about 150 g. were placed in Krebs–Ringer phosphate medium of the following composition: NaCl, 128 mM; $CaCl_2$, 3·6 mM; $MgSO_4$, 1·3 mM; Na_2HPO_4, 10 mM (brought to pH 7·4 with N-HCl). Sodium [1-^{14}C]acetate (200000 counts/min.) was added to the main vessel at the start of the experiment at the concentrations indicated. The usual weight of the slices was 60–70 mg. The final volume of medium in each Warburg manometric flask was 1 ml. Glucose (5 mM) or KCl (100 mM) was added as indicated. The temperature of incubation was 37°. Incubations were carried out in O_2 for 1 hr. The results are means ± S.D. of four independent observations. The radioactivities of $^{14}CO_2$ and ^{14}C-labelled amino acids were estimated as described in the Materials and Methods section. The values for the ^{14}C-labelled amino acids refer to those found in the tissue at the end of the experiment.

Substrates	Final concn. of KCl (mM)	Q_{O_2}	Amounts of $^{14}CO_2$ or ^{14}C-labelled amino acid (mμg.atoms of ^{14}C incorporated/100 mg. wet wt. of tissue/hr.)					
			$^{14}CO_2$	^{14}C-labelled glutamate	^{14}C-labelled glutamine	^{14}C-labelled aspartate	^{14}C-labelled alanine	^{14}C-labelled γ-amino-butyrate
[1-^{14}C]Acetate (1 mM)	5	6·2±0·4	52± 4	18±2	3±2	32±2	<2	3±2
[1-^{14}C]Acetate (10 mM)	5	6·8±0·6	100± 4	48±3	3±1	84±4	<2	<2
[1-^{14}C]Acetate (1 mM) + glucose (5 mM)	5	10·0±0·5	105± 7	53±7	97±8	12±3	<2	8±2
[1-^{14}C]Acetate (1 mM)	105	6·2±0·4	110± 4	28±3	3±1	90±7	<2	<2
[1-^{14}C]Acetate (1 mM) + glucose (5 mM)	105	16·0±0·5	154±11	74±6	86±5	17±3	<2	12±3

Table 2. *Distribution of* ^{14}C*-labelled amino acids between rat brain-cortex slices and medium after incubation with* [1-^{14}C]*acetate* (1mM)

Conditions were as given in Table 1. The results are means ±S.D. of four independent observations.

		Amounts of $^{14}CO_2$ or ^{14}C-labelled amino acids (mμg.atoms of ^{14}C incorporated/100mg. wet wt. of tissue or 0·1ml. of medium/hr.)									
			^{14}C-labelled glutamate		^{14}C-labelled glutamine		^{14}C-labelled aspartate		^{14}C-labelled γ-aminobutyrate	
Incubation medium	Q_{O_2}	$^{14}CO_2$	Tissue	Medium	Tissue	Medium	Tissue	Medium	Tissue	Medium
Krebs–Ringer phosphate + glucose (5mM)	10·6±0·6	105± 7	53±7	0·5±0·2	97±8	1·8±0·3	12±3	<0·2	8±2	<0·2
Krebs–Ringer phosphate + glucose (5mM) + KCl (100mM)	16·1±0·5	154±11	74±6	0·4±0·2	86±5	1·1±0·3	17±3	<0·2	12±3	<0·2

^{14}C-labelled amino acid derived from [1-^{14}C]-acetate (in the absence of added glucose) is aspartate, whose yield exceeds that of the combined yields of ^{14}C-labelled glutamate, glutamine and γ-aminobutyrate. Moreover, the addition of potassium chloride (100mM) markedly increases this yield.

The formation of ^{14}C-labelled aspartate from [1-^{14}C]acetate in the presence of rat brain-cortex slices is to be expected as acetate, presumably even in the absence of added glucose, is metabolized through the citric acid cycle (Black & Kleiber, 1957; Bilinski & McConnell, 1957). Doubtless sufficient oxaloacetate exists in the brain cell from the breakdown of endogenous substrates to enable the citric acid cycle to operate. The relatively high yields of ^{14}C-labelled aspartate compared with that of ^{14}C-labelled glutamate or glutamine may be due to there being insufficient acetyl-CoA available to remove the ^{14}C-labelled oxaloacetate, formed during the citric acid cycle, before transamination takes place.

Only very small quantities of ^{14}C-labelled γ-aminobutyrate are formed from [1-^{14}C]acetate (Tables 1, 2, 4, 5, 6 and 11), the amounts being larger in the presence than in the absence of glucose.

Effect of the addition of glucose on the metabolism of [1-^{14}C]*acetate.* The addition of unlabelled glucose, by its provision of intermediates of the citric acid cycle, causes a fall in the yield of ^{14}C-labelled aspartate from [1-^{14}C]acetate (by transamination with unlabelled α-oxoglutarate) and increased yields of ^{14}C-labelled glutamate, glutamine and γ-aminobutyrate. The latter are due to the formation of oxaloacetate, which, by condensing with ^{14}C-labelled acetyl-CoA, forms eventually ^{14}C-labelled α-oxoglutarate and thereby, by transamination, ^{14}C-labelled glutamate and the derived amino acids.

The stimulation of the rate of formation of $^{14}CO_2$ from [1-^{14}C]acetate in rat brain-cortex slices by the addition of glucose (Table 1), a fact in agreement with the observations of Beloff-Chain *et al.* (1962), may be attributed to an increased rate of formation of ^{14}C-labelled acetyl-CoA by the provision of ATP, and an increased rate of operation of the citric acid cycle.

The enhanced rate of utilization of [1-^{14}C]acetate in the presence of glucose is also shown by the fact that the quantity of [1-^{14}C]acetate remaining in the tissue after the incubation period is smaller than that remaining in the tissue when glucose is absent (Table 3). The total uptake of radioactivity from [1-^{14}C]acetate into the brain-cortex slices in the presence of glucose exceeds considerably that obtaining in the absence of glucose (Table 3), much of the increase being due to the increased quantities of ^{14}C-labelled amino acids that are formed in the presence of glucose and retained in the tissue.

Table 3. *Effects of ouabain and of 2,4-dinitrophenol on the metabolism of* [1-^{14}C]*acetate* (1 mM) *in the presence of rat brain-cortex slices*

Conditions were as given in Table 1. The radioactivities of [1-^{14}C]acetate and of ^{14}C-labelled non-acid-volatile components of tissue and medium were estimated as described in the Materials and Methods section. The results are means ± S.D. of four independent observations.

		Amounts of $^{14}CO_2$, [1-^{14}C]acetate and ^{14}C-labelled non-acid-volatile components in tissue and medium (mμg.atoms of ^{14}C incorporated/100 mg. wet wt. of tissue or 0·1 ml. of medium/hr.)				
			Tissue		Medium	
Additions to Krebs–Ringer phosphate medium	Q_{O_2}	$^{14}CO_2$	[1-^{14}C]-Acetate	^{14}C-labelled non-acid-volatile components	[1-^{14}C]-Acetate	^{14}C-labelled non-acid-volatile components
Glucose (5 mM) present						
None	10·6±0·6	105± 7	28±3	174± 8	77±2	2±0·3
Ouabain (10 μM)	10·6±0·4	21± 3	82±4	30± 3	88±1	3±0·2
KCl (100 mM)	16·1±0·5	154±11	43±5	185±10	72±2	2±0·3
KCl (100 mM)+ouabain (10 μM)	15·3±0·5	90± 8	80±7	45± 5	80±3	3±0·4
2,4-Dinitrophenol (0·1 mM)	10·6±0·3	13± 2	89±9	6± 3	92±2	1±0·2
Glucose absent						
None	7·6±0·5	60± 5	67±8	50± 3	86±2	2·3±0·3
Ouabain (10 μM)	6·7±0·3	21± 3	80±9	20± 3	90±2	2·5±0·3
KCl (100 mM)	6·7±0·5	110±10	44±5	91±10	80±3	3·2±0·4
KCl (100 mM)+ouabain (10 μM)	4·7±0·4	67± 4	67±6	64± 7	84±2	2·3±0·2

Moreover, though the concentration ratio (tissue water/medium) of [1-^{14}C]acetate, at the end of the experiment, is approximately unity in the absence of glucose, it is greatly diminished (to a value of 0·4) in the presence of glucose (Table 3). This fact points to the conclusion that the rate of utilization of acetate in the presence of glucose exceeds that of its transfer into the brain cell, whereas this is not the case in the absence of glucose.

Effects of the addition of potassium chloride (100 mM) to a physiological Ringer medium on the metabolism of [1-^{14}C]*acetate.* A noteworthy finding, sharply differentiating the oxidative metabolism of acetate from that of succinate, is the effect of the addition to the incubation medium of potassium chloride (100 mM). Such an addition markedly increases the rate of $^{14}CO_2$ formation from [1-^{14}C]-acetate both in the presence and absence of glucose (Tables 1 and 3). The addition of potassium chloride (100 mM) has no such effect on the rate of formation of $^{14}CO_2$ from [1,2-^{14}C]succinate (Gonda & Quastel, 1962*a*).

The increased yield of $^{14}CO_2$ in the absence of glucose is not accompanied by an increased respiratory rate (Tables 1 and 3) and is therefore not due to a stimulation of respiration as a whole. The addition of K^+ in the absence of glucose brings about an increased rate of utilization of [1-^{14}C]-acetate, as shown by the fact that the concentration ratio (tissue water/medium) of [1-^{14}C]acetate falls from approximately unity to 0·56 (Table 3) whereas the total uptake of radioactivity by the brain cell from [1-^{14}C]acetate is only slightly increased. The increased utilization, in the presence of additional K^+, is shown particularly by the increased yields of $^{14}CO_2$ and ^{14}C-labelled aspartate (Tables 1 and 3).

In the presence of glucose, the addition of potassium chloride (100 mM) increases the respiratory rate and the rate of evolution of $^{14}CO_2$ from [1-^{14}C]acetate (Tables 1, 2 and 3). It does not change, however, in any significant manner, the yields of ^{14}C-labelled amino acids (Tables 1 and 2) derived from [1-^{14}C]acetate (Table 2).

Effects of 2,4-dinitrophenol on the metabolism of [1-^{14}C]*acetate.* The addition of 2,4-dinitrophenol (0·1 mM) to rat brain-cortex slices incubated in a medium containing glucose and [1-^{14}C]acetate (1 mM) leads to almost complete suppression of the evolution of $^{14}CO_2$ (though the respiratory rate is unchanged) and almost a complete block of acetate utilization (Tables 3 and 4). The concentration ratio (tissue water/medium) of [1-^{14}C]acetate is approximately unity (Table 3). This result is most easily explained by the well-known suppressing action of 2,4-dinitrophenol on ATP formation and hence on the production of ^{14}C-labelled acetyl-CoA from [1-^{14}C]acetate.

Effects of ouabain on the metabolism of [1-^{14}C]-*acetate.* In contrast with the lack of inhibition by ouabain (10 μM) on the rate of $^{14}CO_2$ formation

Table 4. *Effects of ouabain and 2,4-dinitrophenol on the formation of ^{14}C-labelled amino acid from [1-^{14}C]acetate (1 mM) in the presence of rat brain-cortex slices*

Conditions were as given in Table 1. The radioactivities of ^{14}C-labelled amino acids were estimated as described in the Materials and Methods section. The amounts of ^{14}C-labelled alanine found were too small to be measured with accuracy. The results are means ± S.D. of four independent observations.

Additions to Krebs–Ringer phosphate medium	Amounts of ^{14}C-labelled amino acids in tissue (mμg.atoms of ^{14}C incorporated/100 mg. wet wt. of tissue/hr.)			
	^{14}C-labelled glutamate	^{14}C-labelled glutamine	^{14}C-labelled aspartate	^{14}C-labelled γ-aminobutyrate
Glucose (5 mM) present				
None	53±7	97±8	12±3	8±2
Ouabain (10 μM)	15±2	<2	<2	<2
KCl (100 mM)	74±6	86±5	17±3	10±3
KCl (100 mM)+ouabain (10 μM)	35±4	<2	8±2	5±2
2,4-Dinitrophenol (0·1 mM)	4±2	<2	<2	<2
Glucose absent				
None	18±2	3±2	32±2	<2
Ouabain (10 μM)	5±2	3±1	12±2	<2
KCl (100 mM)	22±2	4±2	68±6	<2
KCl (100 mM)+ouabain (10 μM)	14±2	<2	50±4	<2

from [U-^{14}C]glucose by rat brain-cortex slices incubated in a Krebs–Ringer phosphate medium (Gonda & Quastel, 1962*b*), ouabain (10 μM) almost completely suppresses the rate of $^{14}CO_2$ formation from [1-^{14}C]acetate in the presence or absence of glucose (Table 3). Ouabain (10 μM) also suppresses [1-^{14}C]acetate utilization, both in the presence and absence of glucose, the concentration ratio (tissue water/medium) of [1-^{14}C]acetate being approximately unity at the end of the experimental period (Table 3). The rate of formation of ^{14}C-labelled amino acids from [1-^{14}C]acetate is greatly diminished by ouabain (10 μM) (Table 4).

The inhibitory effect of ouabain on the rate of $^{14}CO_2$ formation from [1-^{14}C]acetate is lessened by the presence of potassium chloride (105 mM) (Table 3). An antagonism between ouabain and K^+ is known to take place with adenosine triphosphatase (Dunham & Glynn, 1961) and with rat brain-cortex respiration *in vitro* (Gonda & Quastel, 1962*b*).

The marked action of ouabain in suppressing acetate utilization indicates that this drug affects either the rate of transfer of acetate into the brain cell or the rate of its conversion into acetyl-CoA. It is unlikely that ouabain affects the kinetics of the citric acid cycle, under the given experimental conditions, because there is no diminution of the respiratory rate (Table 3) or of the concentration of the nucleotide pyrophosphates (Table 8 and Gonda & Quastel, 1962*b*) in the brain cell or of the rate of evolution of $^{14}CO_2$ from [U-^{14}C]glucose. For example, in a typical experiment in the presence of rat brain-cortex slices incubated in Krebs–Ringer phosphate medium containing [U-^{14}C]-glucose (5 mM) for 1 hr. at 37°, the evolution of $^{14}CO_2$ amounted to 3470 mμmoles/100 mg. wet wt. of tissue in the absence of ouabain and 3515 mμ-moles/100 mg. in the presence of ouabain (10 μM).

To throw further light on the mode of action of ouabain in suppressing acetate metabolism in isolated brain tissue, experiments were carried out with media containing different concentrations of Na^+ and K^+.

Effects of Na^+ *on acetate metabolism* When rat brain-cortex slices are incubated in a medium containing tris buffer (pH 7·4), tris [1-^{14}C]acetate and glucose, and devoid of Na^+ and K^+, the normal rate of evolution of $^{14}CO_2$ from the [1-^{14}C]acetate is much diminished and this rate is not affected by the addition of ouabain (10 μM) (Table 5). The addition of sodium chloride (100 mM) or potassium chloride (100 mM) increases the rate of $^{14}CO_2$ formation, but only the Na^+-stimulated process is suppressed by the addition of ouabain (10 μM). The addition of both sodium chloride (100 mM) and potassium chloride (100 mM) leads to an optimum rate of respiration and of $^{14}CO_2$ evolution. The latter rate is also suppressed by the addition of ouabain (10 μM) (Table 5). The yield of ^{14}C-labelled glutamine from [1-^{14}C]acetate in a tris buffer is diminished from the normal (compare Tables 1, 5 and 6), this being largely due to the absence of phosphate ions (O. Gonda & J. H. Quastel, unpublished work), and this yield is further diminished by the presence of ouabain (Table 5), as has already been demonstrated when [$^{14}C_6$]glucose is metabolized by rat brain cortex *in vitro* (Gonda & Quastel, 1962*b*).

In the presence of high concentrations of K^+ and

Table 5. *Effects of ouabain on cerebral* [1-^{14}C]*acetate* (1 mM) *metabolism in the presence of various concentrations of* Na^+ *and* K^+

Rat brain-cortex slices were incubated for 1 hr. at 37° in O_2 in an incubation medium containing tris buffer (10 mM) (brought to pH 7·4 with N-HCl), tris [1-^{14}C]acetate (1 mM; 200000 counts/min.) and glucose (5 mM). Sucrose (250 mM) or NaCl (100 mM) or KCl (100 mM) were added where indicated. $CaCl_2$, $MgSO_4$ and Na_2HPO_4 were omitted in these experiments. The final volume of medium in each Warburg manometric flask was 1 ml. The results are means ± S.D. of four independent observations.

Additions					Amounts of $^{14}CO_2$ or ^{14}C-labelled amino acids (mμg.atoms of ^{14}C incorporated/100 mg. wet wt. of tissue/hr.)				
Sucrose (mM)	NaCl (mM)	KCl (mM)	Ouabain (μM)	Q_{O_2}	$^{14}CO_2$	^{14}C-labelled glutamate	^{14}C-labelled glutamine	^{14}C-labelled aspartate	^{14}C-labelled γ-amino-butyrate
250	0	0	0	7·3±0·3	46± 5	23±3	10±2	5±2	3±2
250	0	0	10	7·3±0·3	46± 4	10±2	<2	3±2	<2
0	100	0	0	13·8±0·5	89± 7	33±4	31±3	6±2	6±2
0	100	0	10	10·8±0·7	31± 9	12±2	8±2	<2	2
0	0	100	0	11·0±0·6	118± 9	40±4	<2	25±3	5±2
0	0	100	10	10·6±0·5	120±10	30±4	<2	16±3	<2
0	100	100	0	16·3±0·3	254±25	68±6	22±3	29±4	7±3
0	100	100	10	14·3±0·4	110± 8	55±4	3±2	21±3	4±3

Table 6. *Influence of* Na^+ *and* K^+ *on the distribution of* ^{14}C*-labelled amino acids derived from* [1-^{14}C]*acetate* (1 mM) *between tissue and medium after incubation with rat brain-cortex slices*

Conditions were as given in Table 5. The results are means ± S.D. of four independent observations.

Additions			Amounts of $^{14}CO_2$ or ^{14}C-labelled amino acids (mμg.atoms of ^{14}C incorporated/100 mg. wet wt. of tissue or 0·1 ml. of medium/hr.)							
				^{14}C-labelled glutamate		^{14}C-labelled glutamine		^{14}C-labelled aspartate		^{14}C-labelled γ-aminobutyrate	
NaCl (mM)	KCl (mM)	Q_{O_2}	$^{14}CO_2$	Tissue	Medium	Tissue	Medium	Tissue	Medium	Tissue	Medium
100	0	13·5±0·4	72± 7	23±3	<0·2	31±3	1·8±0·3	7±2	<0·2	6±2	<0·2
100	5	13·6±0·4	91± 6	31±3	<0·2	21±2	2·7±0·4	5±2	<0·2	4±2	<0·2
0	100	10·6±0·5	115± 5	40±5	4·2±0·5	<2	2·2±0·3	14±2	0·3±0·2	7±2	0·3±0·2
5	100	9·7±0·4	117± 8	51±3	3·5±0·4	5±2	2·0±0·3	13±3	0·3±0·2	5±2	0·3±0·2
100	100	16·2±0·3	254±16	68±7	1·8±0·2	22±3	2·6±0·3	15±3	<0·2	7±2	<0·2

low concentrations of Na^+, the distribution of ^{14}C-labelled glutamate between tissue and medium is changed, increased quantities of the amino acid being found in the medium (Table 6). This may be due to a diminished rate of the active transport process for glutamate due to Na^+ lack or possibly to competition between glutamate and K^+ for the carrier site.

Inhibition of acetate oxidation by ouabain is most easily understood as due to suppression by ouabain of an active sodium-transport process, i.e. the sodium pump. Its effect cannot be due to a direct inhibition of the rate of conversion of acetate into acetyl-CoA as ouabain has no inhibitory effect on acetate oxidation in a K^+-rich medium that is devoid of added Na^+ (Table 5). It has therefore to be concluded that ouabain sensitivity of the process of acetate oxidation, in a physiological Ringer medium, is due to its inhibition of a Na^+-dependent carrier-mediated transfer of acetate in a manner already well demonstrated with amino acids.

Effects of sodium fluoroacetate on [1-^{14}C]*acetate metabolism.* The acetate analogue fluoroacetate, at a concentration (1mM) that is without effect on the rates of rat brain-cortex respiration or $^{14}CO_2$ evolution in the presence of [$^{14}C_6$]glucose decreases the rate of $^{14}CO_2$ evolution from [1-^{14}C]acetate to a level that is not influenced by either the presence or absence of Na^+. Typical results are shown in Table 7. These results show that increasing the potassium chloride concentration from 105mM to 333mM in a tris buffer devoid of Na^+ does not increase the rate of respiration or of $^{14}CO_2$ evolution from [1-^{14}C]acetate. The addition of sodium chloride (128mM) to the medium containing potassium chloride (105mM) doubles the respiratory rate and more than doubles the rate of $^{14}CO_2$ evolution. The further addition of potassium chloride to the medium containing sodium chloride (128mM) and potassium chloride (105mM) brings about a diminution of respiratory rate and some diminution also in the rate of $^{14}CO_2$ evolution. In the presence of fluoroacetate (1mM) the addition of sodium chloride has no accelerating effect on the rate of evolution of $^{14}CO_2$ such as is observed in the absence of fluoroacetate.

Sodium fluoroacetate (1mM) exercises no significant diminution of the nucleotide pyrophosphate concentration in the presence of 105mM-potassium chloride when rat brain-cortex slices are incubated in a glucose medium and there is only approximately 20% fall in the concentration in the presence of 5mM-potassium chloride (Table 8). Thus the effects of fluoroacetate cannot be attributed to a significant diminution in the concentration of ATP in the cell. The fall in the nucleotide pyrophosphate concentration due to increased K^+ concentration is dependent on the presence of Na^+ (Table 9).

Effects of sodium propionate and sodium butyrate on the metabolism of [1-^{14}C]*acetate.* The fact that [1-^{14}C]acetate may accumulate in the rat brain cortex *in vitro* against a concentration gradient is shown by the use of sodium propionate or sodium butyrate, each of which suppresses [1-^{14}C]acetate utilization in the tissue. Typical results are shown in Table 10. Sodium propionate at concentrations of 1mM or 10mM greatly inhibits the rate of evolution of $^{14}CO_2$ from [1-^{14}C]acetate in the presence of rat brain-cortex slices incubated in Krebs–Ringer phosphate medium containing glucose (5mM). The concentration ratio (tissue water/medium) for [1-^{14}C]acetate rises above unity, whereas normally the concentration ratio is below unity. Sodium butyrate (1mM and 10mM) also decreases the rate of evolution of $^{14}CO_2$ from [1-^{14}C]acetate, though its effect appears to be less than that of equal concentrations of sodium propionate. The rate of acetate utilization is diminished and a concentration ratio (tissue

Table 7. *Effects of sodium fluoroacetate on the metabolism of* [1-^{14}C]*acetate* (1mM) *at various concentrations of* Na^+ *and* K^+ *in the presence of rat brain-cortex slices*

Conditions were as given in Table 1 except that NaCl was replaced by KCl where indicated and that the phosphate buffer was 1mM-KH_2PO_4 brought to pH7·4 with N-HCl. Glucose (5mM) was present throughout. The results are the means ± S.D. of four independent observations.

Additions		Q_{O_2}		Amounts of $^{14}CO_2$ (mμg.atoms of ^{14}C/100mg. wet wt. of tissue/hr.)	
NaCl (mM)	KCl (mM)	Fluoroacetate absent	Fluoroacetate (1mM) present	Fluoroacetate absent	Fluoroacetate (1mM) present
0	105	8·3±0·4	7·9±0·3	60±3	45±3
0	233	7·9±0·3	7·3±0·3	58±3	44±3
0	333	5·2±0·2	5·0±0·4	49±2	36±2
128	105	16·9±0·6	16·8±0·3	148±4	47±4
128	233	10·7±0·5	11·0±0·5	136±4	41±3
128	333	11·4±0·3	10·3±0·3	125±5	33±2

Table 8. *Effects of ouabain, sodium fluoroacetate and* K^+ *on the incorporation of* ^{32}P *into nucleotide pyrophosphate (ATP and ADP) in rat brain-cortex slices after incubation for* 1 *hr. at* 37°

Conditions were as given in Table 1 except that glucose (5 mM) and purified sodium [^{32}P]phosphate (10^6 counts/min.) were added to, and acetate was omitted from, each vessel. The nucleotide pyrophosphates and the radioactivities were determined as described in the Materials and Methods section. The results are means ± S.D. of six independent determinations.

	Nucleotide pyrophosphate (ATP and ADP)					
	Labelling (counts/min./100 mg. wet wt. of tissue/hr.)		Hydrolysable nucleotide pyrophosphate (mμg.atoms of P/100 mg. wet wt. of tissue/hr.)		Mean specific activities (counts/min./mμg.atom of P)	
K^+ (m-equiv./l.)	5	105	5	105	5	105
Additions						
None	6620±420	6500±550	161±11	85±5	46	76
Ouabain (10 μM)	6720±344	6800±420	160± 7	80±7	42	86
Fluoroacetate (1 mM)	5100±300	5800±400	129±11	80±6	40	72

Table 9. *Effects of changes of* Na^+ *and* K^+ *concentrations on the concentration of the nucleotide pyrophosphates, and on the incorporation of* ^{32}P *into the nucleotide pyrophosphates (ATP and ADP), in rat brain-cortex slices* in vitro

Conditions were as given in Table 1 except that sodium [^{32}P]phosphate (10^6 counts/min.) and glucose (5 mM) were added to each vessel and that acetate was omitted. The results are means ± S.D. of four independent determinations.

Additions		Nucleotide pyrophosphate (ATP and ADP)	
NaCl (mM)	KCl (mM)	Radioactivities (counts/min./100 mg. wet wt. of tissue/hr.)	Hydrolysable nucleotide pyrophosphate (mμg.atoms of P/100 mg. wet wt. of tissue/hr.)
128	5	6840±513	192±11
128	105	6810±493	104± 7
0	105	3600±230	131± 6
0	210	3550±182	135± 7

Table 10. *Effects of sodium propionate and sodium butyrate on the metabolism of* [1-^{14}C]*acetate* (1 mM) *in the presence of rat brain-cortex slices*

Conditions were as given in Table 1 except that KCl (105 mM) was present throughout. The results are means ± S.D. of four independent observations.

		Amounts of $^{14}CO_2$ evolved and [1-^{14}C]acetate and ^{14}C-labelled non-acid-volatile components in tissue and medium (mμg.atoms of ^{14}C incorporated/100 mg. wet wt. of tissue or 0·1 ml. of medium/hr.)					
			[1-^{14}C]Acetate		^{14}C-labelled non-acid-volatile components		Concn. ratio for [1-^{14}C]-acetate (tissue water/ medium)
Additions to Krebs–Ringer phosphate medium containing glucose (5 mM) + KCl (105 mM)	Q_{O_2}	$^{14}CO_2$	Tissue	Medium	Tissue	Medium	
None	16·1±0·5	154±7	43±2	72±6	185±7	2±0·05	0·7
Sodium propionate (1 mM)	15·9±0·3	30±1	130±5	86±7	35±3	0·9±0·1	1·79
Sodium propionate (10 mM)	14·9±0·4	32±1	141±1	85±4	28±3	0·8±0·1	1·96
Sodium butyrate (1 mM)	16·0±0·3	70±3	160±7	77±5	98±5	1·5±0·1	2·48
Sodium butyrate (10 mM)	16·2±0·4	78±4	158±7	76±5	104±5	1·7±0·1	2·48

water/medium) for [1-^{14}C]acetate exceeding 2·0 may be reached (Table 10).

Effects of ammonium chloride on the metabolism of [1-^{14}C]*acetate.* The addition of ammonium chloride to rat brain-cortex slices incubated in a physiological Ringer medium containing [1-^{14}C]-acetate brings about a considerable diminution of the rate of evolution of $^{14}CO_2$, whether glucose is present or not (Table 11). Whereas, as is well known, 10mM-ammonium chloride increases the respiratory rate in the presence of glucose, it decreases the rate in the absence of glucose (Table 11). Ammonium chloride at 100mM inhibits brain respiration in the presence or absence of glucose. The presence of ammonium chloride at 10mM, moreover, diminishes the yield of ^{14}C-labelled amino acids derived from [1-^{14}C]acetate both in the presence or absence of glucose. It should be noted that 10mM-ammonium chloride has no suppressing effect on the yields of ^{14}C-labelled amino acids derived from [U-^{14}C]glucose; in fact, the yield of ^{14}C-labelled glutamine is increased (Gonda & Quastel, 1962*b*).

The diminished utilization of [1-^{14}C]acetate in the presence of ammonium chloride may be attributed to the diminished concentration of ATP in its presence (Acs, Balazs & Straub, 1953). An uncoupling effect of 100mM-ammonium chloride in mitochondrial metabolism has also been observed (Gatt & Racker, 1959; see also Losada & Arnon, 1963).

Effects of glycine on the metabolism of [1-^{14}C]-*acetate.* The addition of glycine (25mM) exercises a decided inhibition of the rate of $^{14}CO_2$ evolution from [1-^{14}C]acetate incubated in a Krebs–Ringer phosphate medium containing glucose (Table 12). It also depresses the rate of utilization of acetate, so that the concentration ratio (tissue water/

Table 11. *Effects of* NH_4^+ *on the metabolism of* [1-^{14}C]*acetate* (1mM) *in rat brain-cortex slices*

Conditions were as given in Table 1. The results are means ±S.D. of four independent observations.

Additions to Krebs–Ringer phosphate medium	Q_{O_2}	Amounts of $^{14}CO_2$ or ^{14}C-labelled amino acids in tissue (mμg.atoms of ^{14}C incorporated/100mg. wet wt. of tissue/hr.)				
		$^{14}CO_2$	^{14}C-labelled glutamate	^{14}C-labelled glutamine	^{14}C-labelled aspartate	^{14}C-labelled γ-amino-butyrate
Glucose (5mM) present						
None	10·6±0·6	105±7	53±7	97±8	12±3	8±2
NH_4Cl (10mM)	12·7±0·3	43±5	22±3	26±3	3±1	5±2
NH_4Cl (100mM)	5·3±0·3	17±2	3±2	<2	<2	<2
Glucose absent						
None	7·6±0·5	60±5	19±3	4±2	30±3	<2
NH_4Cl (10mM)	5·0±0·3	21±2	4±2	<2	8±2	<2
NH_4Cl (100mM)	2·3±0·3	11±3	<2	<2	<2	<2

Table 12. *Effects of glycine on the metabolism of* [1-^{14}C]*acetate* (1mM) *in rat brain-cortex slices*

Conditions were as given in Table 1. Glucose (5mM) was present throughout. Amounts of $^{14}CO_2$ and of [1-^{14}C]acetate and ^{14}C-labelled non-acid-volatile components in tissue and medium are expressed as mμg.atoms of ^{14}C incorporated/100mg. wet wt. of tissue or 0·1ml. of medium/hr. The results are means ±S.D. of four independent determinations.

Concn. of glycine (mM)	0	10	25
Q_{O_2}	10·5±0·4	10·4±0·3	10·4±0·3
$^{14}CO_2$	104±7	96±8	30±4
Tissue			
[1-^{14}C]Acetate	42±3	64±5	83±4
^{14}C-labelled non-acid-volatile components	137±11	103±9	28±3
Medium			
[1-^{14}C]Acetate	78±6	80±5	89±8
^{14}C-labelled non-acid-volatile components	3±0·2	2±0·2	1·5±0·2

medium) of [1-^{14}C]acetate is approximately unity. Whether the glycine acts by depressing the concentration of tissue ATP or by acting as a competing acetate analogue or by some other mechanism is a problem still to be resolved.

DISCUSSION

The facts recorded in this paper show that [1-^{14}C]acetate undergoes metabolism in the presence of rat brain-cortex slices incubated in a Krebs–Ringer phosphate medium at 37° to form $^{14}CO_2$ and ^{14}C-labelled amino acids (glutamate, glutamine, aspartate and relatively small quantities of γ-aminobutyrate).

The rate of oxidation of [1-^{14}C]acetate to $^{14}CO_2$ is approximately doubled by the addition of glucose. The pattern of ^{14}C-labelled amino acids obtained from [1-^{14}C]acetate in the absence of glucose, in which the yield of ^{14}C-labelled aspartate exceeds the combined yield of ^{14}C-labelled glutamate, glutamine and γ-aminobutyrate, is changed by the addition of glucose. Under these circumstances the yield of ^{14}C-labelled aspartate is diminished whereas that of ^{14}C-labelled glutamate and glutamine is increased. These results are explicable on the basis of acetate undergoing oxidation in the isolated brain tissue by operation of the citric acid cycle, the presence of glucose having the double effect of increasing the rate of formation of ^{14}C-labelled acetyl-CoA from [1-^{14}C]-acetate (by provision of ATP) and the rate of formation of oxaloacetate ensuring an increased rate of condensation with acetyl-CoA and therefore of the rate of operation of the citric acid cycle. Thus the presence of glucose brings about an increased rate of utilization of [1-^{14}C]acetate in the isolated brain tissue.

The addition of potassium chloride (100mM) to the incubation medium markedly increases the rate of $^{14}CO_2$ formation from [1-^{14}C]acetate both in the presence and absence of glucose, a phenomenon not accompanied by an increased respiratory rate in the absence of glucose. The evidence points to an increased rate of utilization of acetate secured by the addition of K^+. The effect of K^+ is dependent on the presence of Na^+, for increasing the concentration of potassium chloride from 105mM to 333mM has no effect on the rate of evolution of $^{14}CO_2$ from [1-^{14}C]acetate, whereas the addition of sodium chloride (128mM) to the lowest concentration of potassium chloride doubles the rate of evolution of $^{14}CO_2$ (Table 7). The increased rate of utilization of [1-^{14}C]acetate in the isolated rat brain cortex therefore seems to be dependent on the process of active sodium transport, i.e. on the operation of the sodium pump. It would be logical to conclude that stimulation of the sodium pump by increased K^+ concentration would enhance the rate of acetate transfer into the brain cell and hence its rate of metabolism. An alternative point of view that an increased concentration of K^+ leads to an increased rate of acetate transformation into acetyl-CoA (a process known to be stimulated by K^+ and inhibited by Na^+; Von Korff, 1953) seems less likely, because even with an external potassium chloride concentration of 333mM the rate of $^{14}CO_2$ formation is only the same as with an external concentration of 105mM and is half the value of the rate obtained when Na^+ is added (Table 7). However, such a conclusion does not mean that intracellular K^+ does not play an important role in the conversion of acetate into acetyl-CoA.

The results of the addition of ouabain, which almost completely blocks the rate of $^{14}CO_2$ formation from [1-^{14}C]acetate in the presence or absence of glucose, support the conclusion that transfer of acetate into the brain cell is dependent on the activity of the membrane adenosine triphosphatase and thus on the activity of the sodium pump. This conclusion is based mainly on the observations that ouabain is an effective inhibitor of both the Na^+-plus-K^+-dependent adenosine triphosphatase and the active-transport process (Skou, 1965; Quastel, 1965). It is unlikely that ouabain affects the rate of conversion of acetate into acetyl-CoA for it has no inhibitory effect on [1-^{14}C]acetate conversion into $^{14}CO_2$ in a K^+-rich medium that is devoid of Na^+ (Table 5). Nor is it likely that ouabain inhibits the operation of the citric acid cycle, for in normal physiological media it does not suppress the rate of formation of $^{14}CO_2$ from [$^{14}C_6$]glucose.

The presence of 2,4-dinitrophenol (0·1mM) suppresses the rate of utilization of [1-^{14}C]acetate in the presence of isolated rat brain tissue, and this may be explained by suppression of the rate of acetyl-CoA formation (by diminution of the ATP concentration).

The addition of sodium fluoroacetate (1mM) brings about a considerable diminution in the rate of $^{14}CO_2$ formation from [1-^{14}C]acetate in the presence of isolated brain tissue. It exercises a far larger suppression of the rate of [1-^{14}C]acetate conversion into $^{14}CO_2$ in a medium containing both Na^+ and K^+ than in one lacking Na^+. These results are consistent with the conclusion that fluoroacetate blocks acetate transport at a membrane carrier dependent for its activity on the operation of the sodium pump. Therefore in the presence of fluoroacetate only the passive diffusion of acetate (independent of Na^+ and K^+) takes place. The fact that [1-^{14}C]acetate may accumulate in the rat brain cortex *in vitro* against a concentration gradient is shown by the use of sodium propionate or sodium butyrate, each of which suppresses [1-^{14}C]acetate utilization in the brain tissue.

The results point to the conclusion that acetate transport into the brain cell is a carrier-mediated process, dependent on the active sodium-transport process. Its utilization in the brain is controlled by the activity of the acetate-transport process and also by the rate of its conversion into acetyl-CoA. Acetate utilization is suppressed by NH_4^+ in the brain cell; presumably this is due to diminution of the ATP concentration, on which the rate of conversion of acetate into acetyl-CoA is dependent.

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