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Tricarboxylic Acid-Cycle Metabolism in Brain

EFFECT OF FLUOROACETATE AND FLUOROCITRATE ON THE LABELLING OF GLUTAMATE, ASPARTATE, GLUTAMINE AND γ-AMINOBUTYRATE

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1. The effect of fluoroacetate and fluorocitrate on the compartmentation of the glutamate–glutamine system was studied in brain slices with L-[U-^{14}C]glutamate, L-[U-^{14}C]aspartate, [1-^{14}C]acetate and γ-amino[1-^{14}C]butyrate as precursors and in homogenates of brain tissue with [1-^{14}C]acetate. The effect of fluoroacetate was also studied *in vivo* in mouse brain with [1-^{14}C]acetate as precursor. 2. Fluoroacetate and fluorocitrate inhibit the labelling of glutamine from all precursors but affect the labelling of glutamate to a much lesser extent. This effect is not due to inhibition of glutamine synthetase. It is interpreted as being due to selective inhibition of the metabolism of a small pool of glutamate that preferentially labels glutamine.

Fluoroacetic acid has been established as the toxic principle of the South African plant *Dichapetalum cymosum* that poisons cattle. The mechanism of its action has been established as being due to the 'lethal synthesis' of fluorocitrate which inhibits the operation of the tricarboxylic acid cycle by competitive inhibition of the enzyme aconitase (EC 4.2.1.3) (Peters, 1957). Although administered fluoroacetate produced convulsions, and therefore must affect the central nervous system, the question was raised whether 'lethal synthesis' of fluorocitrate occurred in the brain, or in some other organ and the fluorocitrate carried to the brain (Peters, 1957; Morselli *et al.* 1968).

A study by Lahiri & Quastel (1963) with rat brain slices showed that fluoroacetate strongly inhibited the incorporation of radioactivity from [6-^{14}C]glucose into glutamine but increased the amount of radioactivity in glutamic acid. It also inhibited the transfer of label from [5-^{14}C]glutamate into glutamine. Their observations suggested to us that fluoroacetate may modify the compartmentation of the glutamate–glutamine system in brain.

We have shown that the compartmentation of glutamate metabolism in cerebral cortex may be studied not only *in vivo* but also *in vitro* (Berl, Nicklas & Clarke, 1968). This system lends itself readily to a study of the effects *in vitro* of inhibitors, as well as other substances, on the metabolism of amino acids in brain without having to consider the probable role of the blood–brain barrier or the contribution of other organs.

The present studies with ^{14}C-labelled glutamate, aspartate, acetate and γ-aminobutyrate indicate that fluoroacetate selectively inhibits the tricarboxylic acid cycle associated with the metabolism of the small pool of glutamate that preferentially labels glutamine. The results suggest that 'lethal synthesis' of fluorocitrate does occur in brain. Preliminary reports of this work have been published (Nicklas, Clarke & Berl, 1968; Clarke, Nicklas & Berl, 1970).

METHODS

Experiments in vitro. (*a*) Brain slices. Guinea-pig brain-cortex slices were prepared at room temperature and incubated at 37°C as described by Berl *et al.* (1968). They were preincubated for 10min at 37°C and then transferred to fresh medium before addition of tracer metabolite. Incubation times were 10, 20 and 30min as specified in the tables. Zero time was taken as the point at which the labelled metabolite was added to the medium. The inhibitors were added to both the preincubation and incubation media.

The amino acids from trichloroacetic acid extracts of slices were separated on ion-exchange resin columns, determined and their radioactivities counted as described by Berl *et al.* (1968). When [1-^{14}C]acetate was the labelled metabolite the isolated glutamate and aspartate were

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evaporated to dryness at reduced pressure at 60°C to remove traces of volatile radioactive material and then redissolved in water for determination and counting of radioactivity.

(*b*) Brain homogenates. One hemisphere of guinea-pig cerebral cortex was weighed and homogenized in 5ml of Krebs–Ringer phosphate medium (Umbreit, Burris & Stauffer, 1957) containing 1% of glucose. The homogenization was carried out for approx. 1min in a glass homogenizer with a high-speed-motor-driven tightly fitting Teflon plunger. The homogenate was incubated at 37°C with 12.5μCi of [1-^{14}C]acetate (58μCi/μmol). Portions (1ml) were removed for assay at 10, 20 and 30 min after addition of radioactive metabolite, added to 0.1ml of 50% (w/v) trichloroacetic acid at 0°C and rapidly homogenized. The homogenate was decanted into a plastic centrifuge tube. The homogenizer was washed with 1ml of 5% (w/v) trichloroacetic acid at 0°C and the rinsings were added to the centrifuge tube. After centrifugation the supernatant was treated in the same way as the extract from slices.

Experiments in vivo. Adult mice (Swiss Albino variety) were injected intraperitoneally with 0.1ml of a solution of sodium fluoroacetate in 0.85% NaCl (0.4mg/ml). The dose of fluoroacetate given was 2mg/kg body wt. This dosage is approximately one-half of the LD_{50} reported for rats (Gal, Drewes & Taylor, 1961). After 30min 15μCi of [1-^{14}C]acetate (47μCi/μmol) in 0.1ml of 0.85% NaCl was given intraperitoneally. The mice were killed by decapitation after 5, 10, 20, 40 and 80min. The whole brain was quickly removed (within 1min) and frozen in liquid N_2. The weighed tissue was extracted with 5% (w/v) trichloroacetic acid and processed as described above.

Materials. L-[U-^{14}C]Glutamate, L-[U-^{14}C]aspartate, [1-^{14}C]acetate and γ-amino[1-^{14}C]butyrate were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. L-[1-^{14}C]Glutamate was obtained from Volk Radiochemical Corp., Skokie, Ill., U.S.A. γ-Aminobutyrate was treated by passage through a column of AG 1(X4) resin to remove acidic contaminants; this column was the same type as that used for the separation of the dicarboxylic amino acids. The other labelled compounds were used as supplied since control columns did not indicate cross contamination of amino acid fractions (<0.1%). Sodium fluoroacetate was obtained from Roberts Chemical Co., Nitro, W. Va., U.S.A. It contained less than 1% of acetate as measured by n.m.r. spectroscopy and was calculated to be at least 98% pure by elemental analysis. Barium fluorocitrate was obtained from Calbiochem., Los Angeles, Calif., U.S.A.

RESULTS

In the presence of 1mM-fluoroacetate or 1mM-fluorocitrate and [1-^{14}C]acetate the total radioactivity in the trichloroacetic acid extract of the brain tissue slices was decreased (Table 1). The specific radioactivity of free glutamate was also decreased. In addition, the specific radioactivity of glutamine was lowered to a much greater extent than that of glutamate so that the relative specific radioactivity of glutamate (glutamate = 1) decreased from control values of approx. 3–6 to values of significantly less than 1 (Table 2). This latter relationship did not change significantly when the incubation periods were varied between 10 and 30 min (Table 2). At 0.1mM-fluoroacetate the selective

Table 1. *Effect of fluoroacetate and fluorocitrate on the total radioactivity in the trichloroacetic acid extract and the specific radioactivity of glutamate from* [1-^{14}C]*acetate in guinea-pig brain slices*

The tissue and slices were kept at room temperature until being incubated at 37°C. The medium was Krebs–Ringer phosphate buffer containing 55mM-glucose and 1.55mM-Ca^{2+}. Slices (approx. 100mg) and 2.5ml of medium containing 2.0μCi of [1-^{14}C]acetate (58μCi/μmol) were used. The slices were preincubated at 37°C for 10min and transferred to fresh medium before addition of [1-^{14}C]acetate and incubated for 10–30 min. The Krebs–Ringer phosphate medium used contained sodium fluoroacetate or fluorocitrate both in the preincubation and incubation medium. Total radioactivity refers to total c.p.m. in the trichloroacetic acid extract/g wet wt. of tissue: specific radioactivity of glutamate is in c.p.m./μmol. The results are averages ± S.D.

	Incubation time		
	10min	20min	30min
Control			
10^{-6} × Total radioactivity	1.95±0.98	2.04±0.37	2.22±0.55
10^{-4} × Sp. radioactivity of glutamate	4.69±1.61	8.22±3.29	10.10±2.62
No. of expts.	18	13	12
Fluoroacetate (1mM)			
10^{-6} × Total radioactivity	0.85±0.09	1.05±0.04	1.35±0.12
10^{-4} × Sp. radioactivity of glutamate	1.36±0.01	2.23±0.44	3.16±0.77
No. of expts.	3	3	2
Fluorocitrate (1mM)			
10^{-6} × Total radioactivity	0.85±0.08	1.05±0.05	0.92±0.11
10^{-4} × Sp. radioactivity of glutamate	1.45±0.12	1.82±0.45	1.58±0.03
No. of expts.	3	3	2

Table 2. *Effect of fluoroacetate and fluorocitrate on the relative specific radioactivity of glutamine from* [1-^{14}C]-*acetate in guinea-pig brain slices*

See Table 1 for experimental details. Relative specific radioactivity refers to that relative to glutamate = 1. Results are averages ± S.D. The number of experiments are shown in parentheses except where only a single value was obtained.

	Fluoroacetate			Fluorocitrate		
Incubation time ...	10min	20min	30min	10min	20min	30min
Concn. of inhibitor						
0	5.87±1.38 (18)	4.26±0.76 (13)	3.45±0.43 (12)			
1mM	0.40±0.03 (3)	0.33±0.10 (3)	0.38±0.27 (2)	0.04±0.02 (3)	0.04±0.01 (3)	0.04±0.01 (2)
0.1mM	6.13±2.25 (2)	3.34±1.02 (2)	—	0.08	0.13	—
10μM	6.03±0.08 (2)	5.29±0.76 (2)	—	0.56	0.61	—
1μM	—	—	—	3.96	2.46	—
0.1μM	—	—	—	5.60	4.76	—

Table 3. *Effect of fluoroacetate on the metabolism of labelled amino acids in guinea-pig brain slices*

See Table 1 for experimental details. Slices were incubated with 0.5μCi of L-[U-^{14}C]-glutamate or -aspartate (>200μCi/μmol), 1μCi of L-[1-^{14}C]glutamate (9.5μCi/μmol) or 1μCi of γ-amino[1-^{14}C]butyrate (2.7μCi/μmol) per flask. Relative specific radioactivity refers to that relative to glutamate = 1. Results are averages ± S.D.

	Control			Fluoroacetate (1mM)		
Incubation time ...	10min	20min	30min	10min	20min	30min
L-[U-^{14}C]Glutamate						
10^{-6} × Total radioactivity	2.02±0.37	2.74±0.60	3.13±0.66	2.18±0.20	3.81±0.30	4.62±0.38
10^{-5} × Sp. radioactivity of glutamate	2.01±0.42	2.63±0.69	2.92±0.76	2.08±0.11	3.96±0.58	4.32±0.10
Relative sp. radioactivity of glutamine	1.32±0.33	1.50±0.30	1.67±0.56	0.70±0.04	0.82±0.24	0.86±0.08
No. of expts.	16	14	14	2	2	2
L-[1-^{14}C]Glutamate						
10^{-6} × Total radioactivity	2.92±0.27	4.20±0.27	4.60±0.84	2.98±1.00	3.84±0.14	4.58±0.27
10^{-5} × Sp. radioactivity of glutamate	2.72±0.54	4.55±0.21	4.87±1.21	3.38±0.90	4.62±0.21	5.47±0.28
Relative sp. radioactivity of glutamine	0.97±0.24	1.20±0.56	1.31±0.25	0.49±0.14	0.48±0.04	0.46±0.13
No. of expts.	4	2	2	2	2	2
L-[U-^{14}C]Aspartate						
10^{-6} × Total radioactivity	1.58±0.29	1.72±0.31	2.18±0.44	1.53±0.05	1.79±0.28	2.26±0.05
10^{-4} × Sp. radioactivity of glutamate	2.90±0.69	4.36±0.95	6.72±1.08	2.66±0.38	5.41±0.55	9.03±0.60
Relative sp. radioactivity of glutamine	3.45±0.80	2.49±0.56	2.28±0.47	0.38±0.04	0.23±0.06	0.21±0.01
No. of expts.	12	9	9	2	2	2
γ-Amino[1-^{14}C]butyrate						
10^{-6} × Total radioactivity	1.36±0.53	2.50±0.52	3.08±0.56	1.73±0.28	2.89±0.30	3.58±0.67
10^{-3} × Sp. radioactivity of glutamate	2.04±0.81	5.32±1.56	6.43±1.70	2.14±0.59	4.70±0.89	5.67±1.65
Relative sp. radioactivity of glutamine	4.45±1.22	3.41±0.92	2.57±0.60	0.60±0.13	0.66±0.16	0.54±0.21
No. of expts.	8	8	8	4	4	4

inhibition of the labelling of glutamine was no longer evident and with fluorocitrate this inhibitory effect was evident until its concentration was below 10μM.

Neither of these inhibitors altered significantly the contents of the amino acids in the slices as compared with control values which, in μmol/g wet weight of tissue (±S.D.), were: glutamate, 6.54 ±

Table 4. *Specific radioactivity of glutamate and relative specific radioactivity of glutamine and aspartate in brains of mice poisoned with sodium fluoroacetate and injected with* [1-^{14}C]*acetate*

Each mouse was injected intraperitoneally with 2mg of sodium fluoroacetate/kg in 0.85% NaCl 30min before injection of 15μCi of [1-^{14}C]acetate (0.3μmol) in 0.85% NaCl. At various periods after injection of labelled acetate, mice were decapitated and the brains removed within 1min after death and immediately frozen in liquid N_2. Results are averages ± S.D.

	Incubation time					
	5min	10min	20min	30min	40min	80min
Control						
10^{-5} × Total radioactivity	1.8±0.1			1.5±0.3		
10^{-3} × Sp. radioactivity of glutamate	4.3±0.3			4.2±0.2		
Relative sp. radioactivity of glutamine	2.9±0.2			1.9±0.4		
Relative sp. radioactivity of aspartate	0.7±0.1			0.6±0.1		
No. of expts.	6			6		
Fluoroacetate-treated						
10^{-5} × Total radioactivity	1.6±0.8	2.6±0.5	3.1±1.0		2.7±0.4	0.6±0.3
10^{-3} × Sp. radioactivity of glutamate	6.7±0.5	9.7±0.5	8.2±0.5		7.4±0.4	1.5±0.1
Relative sp. radioactivity of glutamine	1.1±0.2	1.2±0.2	1.8±0.4		1.9±0.3	2.0±0.2
Relative sp. radioactivity of aspartate	0.4±0.2	0.3±0.1	0.4±0.2		0.5±0.2	0.4±0.1
No. of expts.	3	4	4		3	3

Table 5. *Effect of fluoroacetate and fluorocitrate on the specific radioactivity of glutamate and relative specific radioactivity of glutamine labelled from* [1-^{14}C]*acetate in guinea-pig brain homogenates*

Guinea-pig cerebral cortex was homogenized in 5ml of Krebs–Ringer phosphate medium with or without inhibitor. The homogenate was incubated at 37°C with 10μCi of [1-^{14}C]acetate (58μCi/μmol). Samples (1ml) were removed for assay at 10, 20, and 30min after addition of radioactive metabolite. Relative specific radioactivity of glutamine = specific radioactivity of glutamine/specific radioactivity of glutamate. The values are averages for the numbers of experiments given at each time-point. The combined S.D. for the set of specific radioactivities of glutamate measurements was ±72.6 × 10^3 and for the relative specific radioactivity of glutamine ±0.06.

Incubation time ...	10^{-3} × Sp. radioactivity of glutamate (c.p.m./μmol)			Relative sp. radioactivity of glutamine			No. of expts.
	10min	20min	30min	10min	20min	30min	
Control	240.32	322.10	309.97	0.32	0.35	0.46	5
Fluoroacetate							
1mM	38.11	60.60	82.07	0.02	0.03	0.05	3
0.1mM	75.82	120.56	176.46	0.07	0.10	0.12	2
10μM	191.64	309.22	270.53	0.24	0.27	0.42	2
Fluorocitrate							
1mM	10.27	19.06	24.44	0.02	0.03	0.03	1
0.1mM	69.72	78.12	140.55	0.11	0.16	0.13	1
10μM	184.00	294.59	412.92	0.16	0.25	0.30	1
1μM	212.42	279.55	332.75	0.18	0.22	0.23	1

0.75; glutamine, 1.74 ± 0.37; aspartate, 1.31 ±0.23; γ-aminobutyrate, 1.84 ± 0.38; $n = 115$ except for γ-aminobutyrate for which $n = 74$.

With L-[U-^{14}C]glutamate, L-[1-^{14}C]glutamate, L-[U-^{14}C]aspartate or γ-amino[1-^{14}C]butyrate as the labelled precursor 1mM-fluoroacetate had no effect on the total radioactivity in the trichloroacetic acid extract of the brain slices or on the specific radioactivity of free glutamate. The relative specific radioactivity of glutamine, however, was in all cases changed from values greater than 1 to values less than 1 (Table 3).

An effect of fluoroacetate on the labelling of glutamate and glutamine was demonstrated *in vivo* (Table 4). A sublethal dose of fluoroacetate (half the LD_{50} for rats; Gal *et al.* 1961) was administered 30min before the tracer. At 5min after the injection of [1-^{14}C]acetate the relative specific radioactivity

of brain glutamine was less than half that of the control animals. The specific radioactivity of glutamate had increased and that of glutamine had decreased. The relative specific radioactivity of aspartate was also decreased by one-half. The relative radioactivity of glutamine or aspartate approached that of the control animals between 20 and 40 min after administration of tracer. At the dose of inhibitor used in these experiments the animals did not show convulsions or any signs of permanent damage, except for two animals (out of 20) that died. These were not studied. In addition, the amino acid concentrations showed no changes as compared with controls. These latter values, in μmol/g wet wt. ($\pm$S.D.), were: glutamate, 11.25 ± 0.75; glutamine, 4.30 ± 0.45; aspartate, 2.75 ± 0.38; $n = 35$ for each of the three amino acids.

In contrast with slices labelled with [1-^{14}C]-acetate, in guinea-pig brain homogenates the relative specific radioactivity of glutamine was always less than 1 and the specific radioactivity of glutamate was 3–4 times higher than in the slices (Table 5). In the presence of fluoroacetate or fluorocitrate the labelling of glutamic acid was inhibited and that of glutamine even more so, as shown by the markedly decreased relative specific radioactivity values for glutamine. Also in contrast with slices, both inhibitors were equally effective at similar concentrations (Table 5). The concentrations of the amino acids in the control homogenates, in μmol/g wet wt. ($\pm$S.D.) were: glutamate, 9.96 ± 1.34; glutamine, 3.88 ± 0.87; aspartate, 2.74 ± 0.54; γ-aminobutyrate, 2.92 ± 0.39; $n = 24$ for each of these amino acids. Fluoroacetate or fluorocitrate did not significantly alter these values.

DISCUSSION

This study has confirmed the observation by Lahiri & Quastel (1963) that fluoroacetate inhibits incorporation of radioactivity from [^{14}C]glutamate into glutamine. This occurs without significant change in the concentrations of any of the amino acids in the tissue slices. Hence the relative specific radioactivity of glutamine decreases. It reached values of approximately one-half to one-third of the controls and was significantly less than 1 (Table 3). Similar findings were also obtained with [^{14}C]-aspartate, [^{14}C]acetate and γ-amino[^{14}C]butyrate as tracer metabolites (Tables 1, 2 and 3). We have also confirmed the observations of the above authors on the lack of an inhibitory effect of fluoroacetate on purified glutamine synthetase from sheep brain and, in addition, have found fluorocitrate at either 1 mM or 10 mM to be similarly inactive as an inhibitor of glutamine synthetase (D. D. Clarke, W. J. Nicklas & S. Berl, unpublished work). Hence an explanation for the observed inhibition of the labelling of glutamine, other than a direct effect on this enzyme, is needed.

The findings that fluoroacetate and fluorocitrate exert similar inhibition on the labelling of glutamine from [1-^{14}C]acetate in brain slices indicates that the former is probably converted into the latter in this tissue and that both produce their effect by inhibition of the tricarboxylic acid cycle. The paradox that arises here is how the labelling of glutamine can be inhibited 10- to 100-fold compared with its precursor glutamate (Table 2) by a metabolic block at the level of citrate. The concept of metabolic compartmentation, or the presence in brain of more than one tricarboxylic acid cycle, allows a resolution of this paradox (Berl & Clarke, 1969; Berl & Frigyesi, 1968; O'Neal & Koeppe, 1966; Neidle, van den Berg & Grynbaum, 1969). The suggestion has been made that a cycle that is preferentially labelled by glucose and pyruvate is associated with relatively larger pools of tricarboxylic acid cycle intermediates and functions essentially as an 'energy cycle' (Berl, Nicklas & Clarke, 1970). On the other hand, a cycle that preferentially leads to the labelling of glutamine and is associated with relatively smaller pools of tricarboxylic acid cycle intermediates was considered as a 'synthetic cycle'. Fluoroacetate seems to be converted into fluorocitrate and to produce its inhibitory effect largely in this 'synthetic cycle'. This would allow us to explain how these inhibitors can have a profound effect on the labelling of glutamine without a corresponding effect on the labelling of glutamate from a wide variety of labelled precursors. In addition, the inhibitor effect of fluoroacetate on conversion of L-[1-^{14}C]glutamate or L-[U-^{14}C]-glutamate into glutamine suggests that a small pool of ATP probably provides the energy for the amidation of a small pool of 'active' glutamate, which is used for glutamine synthesis; this ATP is intimately associated with the 'synthetic' cycle and its production is therefore selectively inhibited by fluoroacetate.

Further, inhibition of the production of $^{14}CO_2$ from [1-^{14}C]acetate but not from [6-^{14}C]glucose by fluoroacetate (Gonda & Quastel, 1966) agrees very well with this interpretation of at least two tricarboxylic acid cycles. Thus if the block in citrate metabolism by fluorocitrate formation occurred essentially in the smaller 'synthetic cycle', the effective increase in the citrate concentration in that cycle would be many times greater than would be apparent from a measurement of the total tissue content of citrate. It would also have a negligible effect on the total oxygen uptake by the tissue, or on $^{14}CO_2$ production from [^{14}C]glucose, since the latter is oxidized mainly via the larger 'energy cycle'. If acetate and fluoroacetate are both activated mainly in the 'synthetic cycle', then inhibition of $^{14}CO_2$

formation from labelled acetate (Gonda & Quastel, 1966) as well as selective inhibition of glutamine synthesis by fluoroacetate becomes readily explainable. An additional consequence of this picture is that only a small proportion of the fluoroacetate may need to be converted into fluorocitrate to produce a very significant inhibition of this cycle. This may explain the failure of some attempts to demonstrate the formation of fluorocitrate from fluoroacetate by brain tissue (Peters & Shorthouse, 1966; Morselli *et al.* 1968).

Although there has been some controversy as to whether fluoroacetate administered *in vivo* causes elevated citrate concentrations in brain (Peters, 1957) the work of Goldberg, Passonneau & Lowry (1966) leaves little doubt that this does occur in the mouse. However, these authors observed at the same time that the concentrations of ATP and creatine phosphate remain normal, an observation that also may be explained by the compartmented operation of the tricarboxylic acid cycle.

In the present studies *in vivo*, after the administration of sublethal doses of fluoroacetate the relative specific radioactivity of glutamine from [^{14}C]acetate decreased to one-half of the value for control animals. Therefore, fluoroacetate would appear to affect preferentially the small pool of glutamate in the animal as well as in slices.

Although the labelling of glutamine relative to glutamate from [^{14}C]acetate in homogenates of brain tissue is considerably less than in slices the selective inhibition by fluoroacetate or fluorocitrate of the flow of radioactivity into glutamine is still observed (Tables 4 and 5). Since we have suggested that the selective inhibition of the labelling of glutamine by these compounds is due to the compartmentation of the tricarboxylic acid cycle we must conclude that at least in part it survives the disruption of the cells and suggest that it occurs at the mitochondrial level. This would agree with the reported heterogeneity of brain mitochondria (Salganicoff & Koeppe, 1968; Neidle *et al.* 1969) if such heterogeneity applies not only to enzyme distribution but also to the concentrations of the intermediates of the tricarboxylic acid cycle, particularly citrate.

In brain homogenates fluoroacetate and fluorocitrate are equipotent, in contrast with slices where the potency is greater by at least two orders of magnitude. At 0.1 mM-fluoroacetate a distinct effect is observed in homogenates but not in slices. It seems that the entry of fluoroacetate is restricted by an intact cell membrane. In agreement with Gonda & Quastel (1966) the entry of radioactive acetate into brain slices is inhibited by fluoroacetate, which suggests the operation of a carrier-mediated mechanism. The far greater effect of fluorocitrate than of fluoroacetate at low concentrations (10 μM) with intact cells would indicate that the latter is only poorly converted into the former. This would explain the observation by Morselli *et al.* (1968) that fluorocitrate is 100 times as toxic as fluoroacetate when injected intracerebrally. The decreased specific radioactivity of glutamic acid from [^{14}C]-acetate in the presence of these inhibitors is attributed to the competitive inhibition of entry into the cells (Table 1). This conclusion is supported by the fact that such inhibition is not observed with other precursors (Table 3).

The hypothesis that more than one tricarboxylic acid cycle is operative in brain has been used to resolve many of the apparently paradoxical findings reported here and elsewhere about the toxic effects of fluoroacetate and fluorocitrate. It is suggested that a similar explanation may prove helpful in understanding the effects of other metabolic inhibitors.

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