

A Simulation Study of Brain Compartments

METABOLISM OF GLUTAMATE AND RELATED SUBSTANCES IN MOUSE BRAIN

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A computer model of the metabolism of glutamate, glutamine, γ -aminobutyrate, and the tricarboxylic acid cycle in mouse brain has been constructed in terms of 39 reactions among 19 substances or groups of substances (permitting manipulation of 30 independent variables). The model is divided into two compartments, in conformity with previous models based on indirect evidence, and it is found that this compartmentation is indeed the same as that indicated directly with specifically ^{14}C -labelled acetate and glucose. The movement of materials between the large and small compartments has been studied; glutamine appears to flow from the small to the large compartment, γ -aminobutyrate in the reverse direction.

The extent to which [^{14}C]glutamate is converted into glutamine in brain greatly depends on its carbon source. Glutamine is highly labelled relative to glutamate with precursors such as bicarbonate, acetate, aspartate and leucine, but less labelled with glucose (reviewed by Berl & Clarke, 1969). These observations are explainable by the presence in brain of at least two glutamate pools, with two distinct tricarboxylic acid cycles. Glutamine is formed mainly from the smaller of these pools (O'Neal & Koeppe, 1966; Van den Berg, Mela & Waelsch, 1966; Van den Berg, Kržalić, Mela & Waelsch, 1969). The enormous complexities of these metabolic pathways have encouraged the use of computer simulation methods to provide a quantitative picture of the entire system (Garfinkel, 1962, 1966). Previous simulations based mainly on data from rats and cats (with bicarbonate, glutamate, aspartate and [^{15}N]ammonia as precursors) led to the formulation of a model containing two tricarboxylic acid cycles (Garfinkel, 1966).

Fairly detailed data for the time course of incorporation of ^{14}C -labelled glucose and acetate into glutamate, glutamine and aspartate is now available for mice (Van den Berg *et al.* 1966, 1969). We have performed a computer simulation of this data, primarily to find the simplest model that would explain this data as an aid in interpreting it. This approach differs from that in earlier studies (Garfinkel, 1966), in which attempts were made

to explain all available literature data for the several precursors in different animals. It yields a simpler and less sophisticated model, but one based entirely on data from one source (avoiding problems in combining data from different sources). However, the compartmentations indicated by the two models are concordant.

METHODS

The model reported here was constructed using the chemical simulation language of Garfinkel (1968). Reactions representing carbon atom transitions for all metabolites were written in a standard form: $\text{SSUCC}^*1 \rightleftharpoons \text{SFUM}^*1$, $\text{SSUCC}^*2 \rightleftharpoons \text{SFUM}^*2$, ... for all carbon atoms in the molecule. The asterisk indicates a labelled carbon atom and the number its position. The prefixes 'S' or 'L' distinguish between metabolites of small and large compartments. The model is defined by Tables 1 and 2 and summarized in Scheme 1. The reactions in Table 2 are translated into differential equations and solved for radioactivities as a function of time by the simulation program (Garfinkel, 1968). Calculation of the model is sufficiently slow as to render the customary automatic procedures for optimization by least squares economically impossible, as they require many repetitions of such calculations, so that a rigorously defined 'best fit' may not have been obtained. The simulation was performed on the PDP-6 computer of the University of Pennsylvania Medical School Computer Facility.

Rate constants (identical for otherwise identical reactions with different labelling) were determined by manipulating the model to match the experimental data, subject to the constraint that unlabelled concentrations must hold steady state values (checked with an unlabelled version of the model). Radioactive substrates were input to the atom-by-atom model by using continuous input

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Table 1. *List of the compounds used in the model and their concentrations.*

Prefixes L and S denote compartmentation: L for the large compartment; S for the small compartment. When several actual chemicals are combined this is indicated in parentheses. Together with Table 2 this defines the model.

Compound	Concentration ($\mu\text{mol/g}$ wet wt.)
GLUCOSE	2.0
PYRUVATE (pyruvate and lactate)	1.6
L-ACETYL-COA	0.0025
L-CITRATE (citrate, isocitrate and cis-aconitate)	0.35
L-ALPHA-KETOGLUTARATE	0.110
L-SUCCINATE (succinate, fumarate and malate)	1.128
L-OXALOACETATE	0.003
L-GLUTAMATE-1	1.000
L-GLUTAMATE-2	6.000
L-ASPARTATE	2.250
S-ACETYL-COA	0.0025
S-CITRATE (citrate, isocitrate and cis-aconitate)	0.026
S-ALPHA-KETOGLUTARATE	0.015
S-SUCCINATE (succinate, fumarate and malate)	0.090
S-OXALOACETATE	0.001
S-GLUTAMATE-3	1.250
S-ASPARTATE	0.350
GLUTAMINE	3.000
GAMMA-AMINOBYTRATE	1.6

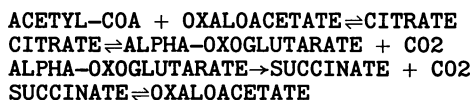
profiles and/or pulse labelling. The glucose input was finally shaped so as to correspond to the observed time-course of glucose labelling in the brain, by using as input a curve constructed from the time derivative of the observed radioactivity (Dzubow & Garfinkel, 1970). Acetate was assumed to be 100% labelled at zero time, i.e. 'pulse' labelling.

Assumptions and simplifications. The model consists of glycolysis and two tricarboxylic acid cycles. Glucose was a precursor of both cycles, acetate of only one. All the individual carbon atoms were traced through the model. The number of equations of the type shown in Table 2 required to represent all reactions among the tricarboxylic acid-cycle intermediates and glutamate pools in this way is greater than 400 (Dzubow & Garfinkel, 1970). In order to permit economical calculation we substantially simplified this model, as follows.

Glycolysis was condensed to: $\text{GLUCOSE} \rightarrow \text{PYRUVATE}$, where PYRUVATE is the sum of pyruvate and lactate. The glycolytic intermediate pool in brain is small compared with the glucose flux (Lowry, Passonneau, Hasselberger & Schulz, 1964), and the lactate dehydrogenase reaction appears far faster than the conversion of pyruvate into acetyl-CoA (Van den Berg *et al.* 1969).

The tricarboxylic acid cycle is represented by four reactions: the entrance of acetyl-CoA, decarboxylation of citrate and α -oxoglutarate, and the regeneration of

oxaloacetate. In roughly computer language (Garfinkel, 1968) these are:



Here CITRATE includes isocitrate and aconitate; SUCCINATE includes fumarate and malate [in a more detailed model (Garfinkel, 1970) these are found to equilibrate within 5 min]. Glutamate and aspartate are connected to the cycle by: $\text{ALPHA-OXOGLUTARATE} \rightleftharpoons \text{GLUTAMATE}$ and $\text{OXALOACETATE} \rightleftharpoons \text{ASPARTATE}$. CO_2 fixation was represented by: $\text{OXALOACETATE} \rightleftharpoons \text{PYRUVATE} + \text{CO}_2$.

The concentration of tricarboxylic acid-cycle intermediates in mouse brain is approximately $1.7 \mu\text{mol/g}$ wet wt. (Goldberg, Passonneau & Lowry, 1966), and about $1.5 \mu\text{mol/g}$ wet wt./min of acetyl CoA enters the cycle (Lowry *et al.* 1964). As the first data point was 5 min after precursor injection (Van den Berg *et al.* 1969) these simplifications will not seriously disturb the calculations. It should also be noted that the pool sizes of glutamate, aspartate and glutamine total about $20 \mu\text{mol/g}$ wet wt., more than 10-fold greater than the total cycle intermediates.

The glutamate pool in the compartment which metabolizes most of the glucose (the large compartment) was assumed to consist of two sub-pools (Garfinkel, 1970); this assumption was not made for the other compartment because its two sub-pools equilibrate rapidly (Garfinkel, 1966). γ -Aminobutyrate was assumed to be formed in the large compartment (C. J. Van den Berg, unpublished work), and degraded in the small compartment, as it has been shown that γ -aminobutyrate gives rise to high relative specific radioactivities of glutamine *in vivo* and *in vitro* (Baxter, 1968; Nicklas, 1968; Balazs, Machiyama, Hammond, Julian & Richter, 1970). The rate of net CO_2 fixation equals the efflux of amino acids from the system, approximately 10% of the rate of acetyl-CoA utilization. About the same amount of the radioactivity in the brain was present in the trichloroacetic acid-insoluble fraction starting with [^{14}C]glucose (C. J. Van den Berg, unpublished work). Lipid formation was not considered. The data used here are from experiments with fed animals, which do differ from starved ones (Van den Berg *et al.* 1969).

Model finally adopted. The model as described, immediately simulated qualitatively the shape of the time-course of labelling of glutamate, glutamine and aspartate by glucose and acetates, and the [^{14}C]glucose/[^{14}C]glucose and [^{14}C]acetate/[^{14}C]acetate labelling ratios.

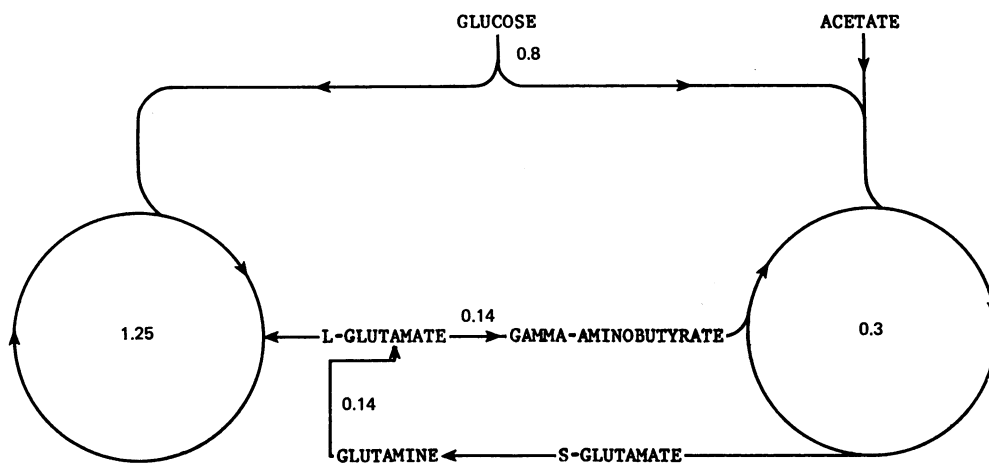
After considerable trial and error, we arrived at model parameters which matched the observed data reasonably closely (except for the 5 min point with glucose). In this process, rate constants were manipulated more often than pool sizes, which should be regarded as less accurately fixed. In Tables 3 and 4 the calculated values are compared with observed values. Generally the fit for glucose was better than that for acetate. However, it was observed that acetate is converted *in vivo* to glucose which then penetrates the brain (Van den Berg *et al.* 1969; C. J. Van den Berg, unpublished work). Altering the input to the model accordingly improved its behaviour (Table 5).

Table 2. List of the reactions in the model and the final values of their fluxes

Together with Table 1, this defines the model.

Reaction	Flux ($\mu\text{mol/g wet wt./min}$)	
	Forward reaction	Backward reaction
GLUCOSE \rightarrow PYRUVATE	0.808	
PYRUVATE \rightarrow L-ACETYL-COA	1.246	
PYRUVATE \rightarrow S-ACETYL-COA	0.301	
PYRUVATE \rightarrow CO ₂	1.547	
PYRUVATE + CO ₂ \rightleftharpoons L-OXALOACETATE	0.080	0.044
L-ACETYL-COA + L-OXALOACETATE \rightarrow L-CITRATE	1.246	
L-CITRATE \rightleftharpoons L-ALPHA-KETOGLUTARATE + CO ₂	1.346	0.100
L-ALPHA-KETOGLUTARATE \rightleftharpoons L-GLUTAMATE-1	20.020	20.000
L-GLUTAMATE-1 \rightleftharpoons L-GLUTAMATE-2	10.000	10.000
L-GLUTAMATE-1 \rightarrow GAMMA-AMINOBUTYRATE	0.137	
L-GLUTAMATE-1 \rightleftharpoons GLUTAMINE	0.075	0.212
L-GLUTAMATE-1 \rightarrow (leaves the system)	0.020	
L-ALPHA-KETOGLUTARATE \rightarrow L-SUCCINATE	1.229	
L-SUCCINATE \rightleftharpoons L-OXALOACETATE	5.000	3.777
L-OXALOACETATE \rightleftharpoons L-ASPARTATE	2.520	2.500
L-ASPARTATE \rightarrow (leaves the system)	0.020	
PYRUVATE + CO ₂ \rightleftharpoons S-OXALOACETATE	0.101	0.067
S-ACETYL-COA + S-OXALOACETATE \rightarrow S-CITRATE	0.300	
S-CITRATE \rightleftharpoons S-ALPHA-KETOGLUTARATE + CO ₂	0.401	0.100
S-ALPHA-KETOGLUTARATE \rightarrow S-GLUTAMATE-3	0.484	0.313
S-GLUTAMATE-3 \rightleftharpoons GLUTAMINE	1.200	1.030
GLUTAMINE \rightarrow (leaves the system)	0.033	
S-ALPHA-KETOGLUTARATE \rightarrow S-SUCCINATE	0.130	
GAMMA-AMINOBUTYRATE \rightarrow S-SUCCINATE	0.137	
S-SUCCINATE \rightleftharpoons S-OXALOACETATE	0.324	0.057
S-OXALOACETATE \rightleftharpoons S-ASPARTATE	0.150	0.150
ACETATE \rightarrow S-ACETYL-COA		

50% of the acetate converted in 1 min

Scheme 1. Overall scheme for the model here described, detailed in Table 2. Values are fluxes in $\mu\text{mol/g wet wt./min}$.

RESULTS

Critical reactions of the model

1. *Glucose utilization.* Lowry & Passonneau (1964) estimated the rate of glucose utilization in

mouse brain *in vivo* to be approximately $0.75/\mu\text{mol/g wet wt./min}$. In rat brain *in vivo* a value of $0.96 \mu\text{mol/g wet wt./min}$ was reported by Gaitonde (1965). By varying the rate of glucose utilization, starting from $0.5 \mu\text{mol/g wet wt./min}$, we found that

Table 3. Comparison of observed and calculated data for the labelling of glutamate, glutamine and aspartate with [1-¹⁴C]glucose and [2-¹⁴C]glucose

The observed data are for starved mice (Van den Berg *et al.* 1969). Standard deviations for observed values are about 5% of the observed value for aspartate and 25–35% for the other substances.

Precursor	Time ...	5 min		10 min		15 min	
		Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
Sp. radioactivity ratio of glucose/glutamate							
[1- ¹⁴ C]Glucose		—	—	1.8	2.2	1.0	0.8
[2- ¹⁴ C]Glucose		9.6	10.8	3.6	2.7	1.5	1.3
Sp. radioactivity of glutamate (%) (30 min=100%)							
[1- ¹⁴ C]Glucose		20	12	71	63	100	100
[2- ¹⁴ C]Glucose		29	19	97	83	100	100
Sp. radioactivity ratio of aspartate/glutamate							
[1- ¹⁴ C]Glucose		0.56	0.62	0.88	0.87	0.92	0.95
[2- ¹⁴ C]Glucose		0.57	0.62	0.85	0.81	0.80	0.80
Sp. radioactivity ratio of glutamine/glutamate							
[1- ¹⁴ C]Glucose		0.32	0.42	0.50	0.61	0.62	0.74
[2- ¹⁴ C]Glucose		0.37	0.42	0.63	0.68	0.88	0.90
% of ¹⁴ C in C-1 of glutamate							
[2- ¹⁴ C]Glucose		9.4	4.7	14.9	16.7	27.3	27.1
% of ¹⁴ C in C-1 of glutamine							
[2- ¹⁴ C]Glucose		2.6	5.0	15.0	10.1	19.5	17.6
[1- ¹⁴ C]Glucose/[2- ¹⁴ C]glucose labelling ratio for							
Glutamate		1.34	1.04	1.38	1.26	1.87	1.64
Glutamine		1.25	1.03	1.15	1.13	1.35	1.34
Aspartate		1.27	1.04	1.41	1.35	2.16	1.93

Table 4. Comparison of observed and calculated data for the labelling of glutamate, glutamine and aspartate with [1-¹⁴C] and [2-¹⁴C]acetate

The observed data are from Van den Berg *et al.* (1969). These authors do not indicate the error in these data.

Precursor	Time ...	5 min		10 min		15 min	
		Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
Sp. radioactivity ratio of aspartate/glutamate							
[1- ¹⁴ C]Acetate		0.46	0.35	0.59	0.48	0.47	0.52
[2- ¹⁴ C]Acetate		0.44	0.54	0.70	0.68	0.70	0.84
Sp. radioactivity of glutamine/glutamate							
[1- ¹⁴ C]Acetate		4.81	4.23	3.27	3.00	2.45	2.31
[2- ¹⁴ C]Acetate		3.55	3.89	1.46	2.40	1.17	1.49
Sp. radioactivity of glutamine (%)							
[1- ¹⁴ C]Acetate		88	91	65	58	37	23
(5 min=100% for [2- ¹⁴ C]acetate)							
[2- ¹⁴ C]Acetate		100	100	74	71	51	34
% of ¹⁴ C in C-1 of glutamate							
[1- ¹⁴ C]Acetate		14.1	10.8	33.1	23.1	38.3	37.5
% of ¹⁴ C in C-1 of glutamine							
[1- ¹⁴ C]Acetate		7.6	9.0	18.7	14.1	27.9	22.7
[2- ¹⁴ C]acetate/[1- ¹⁴ C]acetate labelling ratio for							
Glutamate		1.53	1.20	1.95	1.53	3.47	2.33
Aspartate		1.46	1.83	2.27	2.19	4.30	3.79
Glutamine		1.11	1.10	0.90	1.23	1.66	1.50

a rate of 0.8 $\mu\text{mol/g}$ wet wt./min was necessary to obtain sufficient label early enough in the amino acid pool and to get a large enough difference in amino acid labelling between [1-¹⁴C]- and [2-¹⁴C]-glucose 'experiments'.

2. Acetyl-CoA utilization in both compartments.

Glutamine was assumed to be formed primarily in the small compartment; this assumption determines the rate of glucose utilization in both compartments. In addition, the acetate data gave an estimate of the amount of acetyl-CoA entering the cycle in the small compartment only. By com-

binning these we arrived at values of 0.30 and 1.25 $\mu\text{mol/g wet wt./min}$ for the rate of acetyl-CoA utilization in the small and large compartments respectively.

3. Tricarboxylic acid-cycle reactions and CO_2

Table 5. Results of a model experiment with $[1-^{14}\text{C}]$ and $[2-^{14}\text{C}]$ acetate, in which the labelling of glucose by acetate was taken into account

It was assumed that $[1,2,5,6-^{14}\text{C}]$ glucose was formed from $[2-^{14}\text{C}]$ acetate, and $[3,4-^{14}\text{C}]$ glucose from $[1-^{14}\text{C}]$ acetate. The data on the conversion of acetate to glucose were obtained in the same study as all the data simulated in this paper (Van den Berg *et al.* 1969; C. J. Van den Berg & L. J. Kržalić, unpublished work). These authors do not indicate the error in this data.

Time (min)	Sp. radioactivity ratio of glutamine/ glutamate, $[2-^{14}\text{C}]$ acetate as precursor		
	Observed	Calculated (without correction)	Calculated (with correction)
5	3.55	3.89	3.74
15	1.46	2.40	2.06
30	1.17	1.49	1.25
		[$2-^{14}\text{C}$]acetate/[$1-^{14}\text{C}$]acetate labelling ratio for glutamate	
5	1.53	1.20	1.25
15	1.95	1.53	1.93
30	3.47	2.33	3.76

fixation. The extent of reversibility of the first two cycle reactions, the formation of citrate and its conversion into α -oxoglutarate, was not critical in the range tested (up to 30% back reaction). The situation is different for the conversion of succinate into oxaloacetate. ^{14}C from $[2-^{14}\text{C}]$ glucose can reach C-2 and C-3 of glutamate only by conversion of $[2-^{14}\text{C}]$ pyruvate into $[2-^{14}\text{C}]$ oxaloacetate, which is converted reversibly into a symmetrical intermediate (SUCCINATE). O'Neal & Koeppe (1966) found relatively little ^{14}C in the C-2 plus C-3 positions (which they did not distinguish), indicating that this sequence is not very fast. The rate of equilibration of SUCCINATE and oxaloacetate was set so that labelling of C-3 in glutamate was approximately half that of C-2. The rate of CO_2 fixation was set so as to obtain approximately 10% of the label in glutamate in C-2 plus C-3 (O'Neal & Koeppe, 1966).

4. Relation of glutamate and aspartate with tricarboxylic acid-cycle intermediates. The rate of glutamate labelling appeared to be greatly dependent upon the rate of exchange between α -oxoglutarate and glutamate, as compared with the cycle flux. The ratio of aspartate labelling, as compared with glutamate labelling, also appeared to be very sensitive to the values of these two rates (aspartate being labelled too rapidly at low rates). Some trials are summarized in Table 6, indicating that the exchange between α -oxoglutarate and glutamate should be about 10-fold the cycle flux (which would

Table 6. Effect of different rates of conversion between keto acids and amino acids and between the two large glutamate pools on the rate of labelling of aspartate relative to glutamate

The precursor was $[2-^{14}\text{C}]$ glucose. Fluxes are given in $\mu\text{mol/g wet wt./min}$; all other reaction rates as given in Table 2.

	Final value	Alternatives tested		Observed values
A. Reactions and fluxes				
L-ALPHA-KETOGLUTARATE \rightarrow L-GLUTAMATE-1	20.02	10.02	20.02	
L-GLUTAMATE-1 \rightarrow L-ALPHA-KETOGLUTARATE	20.00	10.00	20.00	
L-OXALOACETATE \rightarrow L-ASPARTATE	2.52	2.52	5.02	
L-ASPARTATE \rightarrow L-OXALOACETATE	2.50	2.50	5.00	
Relative sp. radioactivity of aspartate to that of glutamate at time min ...	Calculated from above fluxes			
5	0.62	0.67	0.68	0.57
15	0.81	0.83	0.83	0.85
30	0.80	0.80	0.80	0.80
B. Reactions and fluxes				
L-GLUTAMATE-1 \rightarrow L-GLUTAMATE-2	10.0	5.0	20.0	
L-GLUTAMATE-2 \rightarrow L-GLUTAMATE-1	10.0	5.0	20.0	
Relative sp. radioactivity of aspartate to that of glutamate at time (min) ...	Calculated from above fluxes			
5	0.62	0.69	0.57	0.57
15	0.81	0.80	0.80	0.85
30	0.80	0.80	0.80	0.80

account for nearly all the glutamate labelling) and different from that for the oxaloacetate-aspartate exchange. Most of the glutamate is present in the large compartment, and much of it must equilibrate rapidly with α -oxoglutarate. The massive incorporation of glucose radioactivity into glutamate also indicates rapid exchange between the two glutamate pools in the large compartment. However, the work of Patel, Balazs & Richter (1970), who found the smaller of these pools 57% more highly labelled after 10 min., does argue for a slower exchange than that arrived at here.

5. *Rate of glutamine and γ -aminobutyrate movements between the two compartments.* Numerical parameters which fitted the observed data were easily found following the assumptions (discussed below) of glutamine flow from the small to the large compartment and the reverse and equal flow of γ -aminobutyrate. A glutamine flow of $0.137 \mu\text{mol/g wet wt./min}$ was found to be adequate and better than 0.1 and 0.160 respectively. This is about twice the value found by Dzubow & Garfinkel (1970) for the rat, but there is a general tendency for the flows reported here to be faster than they found, presumably due to species differences. The $[2\text{-}^{14}\text{C}]\text{-acetate}/[1\text{-}^{14}\text{C}]\text{-acetate}$ labelling ratio for glutamate increases so rapidly with time, because the contribution of glutamate from the large compartment is so large. The small compartment glutamate behaves like glutamine.

DISCUSSION

The essential features of the model described in this paper are the following. 1. There are two tricarboxylic acid cycles, which are the same two indicated directly by the labelling patterns starting from specifically ^{14}C -labelled acetate (Van den Berg *et al.* 1969). 2. These two tricarboxylic acid cycles are connected by glutamine and γ -aminobutyrate.

The presence of two tricarboxylic acid cycles in the brain is based on an analysis of the incorporation of a large number of precursors into glutamate and glutamine (review, Berl & Clarke, 1969) and on previous simulation studies (Garfinkel, 1966). There are two categories of glutamate and glutamine precursors: (1) glucose, glycerol and lactate, primarily incorporated into glutamate (O'Neal & Koeppe, 1966; Van den Berg *et al.* 1969); (2) acetate and leucine (via acetyl-CoA) (O'Neal & Koeppe, 1966; Van den Berg *et al.* 1969; Berl & Frigyesi, 1969); γ -aminobutyrate and propionate (via succinate) (Baxter, 1968; O'Neal, Koeppe & Williams, 1966), primarily incorporated into glutamine. In addition the differences between glucose and acetate labelling patterns suggest the existence of two separate pathways from acetyl-CoA to α -oxo-

glutarate; this extends through to oxaloacetate because γ -aminobutyrate and propionate behave like acetate. Also, the literature on enzyme distribution indicates the existence of at least two different mitochondrial populations, one rich in such enzymes as succinate dehydrogenase (Neidle, Van den Berg & Grynbaum, 1969; Salganicoff & DeRobertis, 1965) and glutaminase (Salganicoff & DeRobertis, 1965; Blokhuis, 1970), and the other rich in acetyl-CoA synthetase (Neidle *et al.* 1969), γ -aminobutyrate transaminase (Van Kempen, Van den Berg, Van der Helm & Veldstra, 1965) and glutamate dehydrogenase (Neidle *et al.* 1969), which are necessary in the metabolism of such glutamine precursors as acetate, γ -aminobutyrate and ammonia.

The γ -aminobutyrate pathway has been considered to be a simple shunt of the tricarboxylic acid cycle, i.e., equimolar amounts of γ -aminobutyrate are formed from glutamate and degraded to succinate; but it is questionable if the glutamate is associated with the same cycle as the succinate. The main arguments are: (a) the rapid labelling of γ -aminobutyrate by glucose (Gaitonde, Dahl & Elliott, 1965; Minard & Mushahwar, 1966); (b) the slow labelling of γ -aminobutyrate by glutamate, aspartate and leucine (Berl, Lajtha & Waelsch, 1961; Berl & Clarke, 1969); (c) the similarity of the $[1\text{-}^{14}\text{C}]\text{-glucose}/[2\text{-}^{14}\text{C}]\text{-glucose}$ and $[2\text{-}^{14}\text{C}]\text{-acetate}/[1\text{-}^{14}\text{C}]\text{-acetate}$ labelling ratios of γ -aminobutyrate with those for glutamate rather than for glutamine (C. J. Van den Berg, unpublished work). These arguments collectively suggest that γ -aminobutyrate is formed primarily by a reaction sequence such as the following: large glutamate pool \rightarrow γ -aminobutyrate \rightarrow succinate \rightarrow α -oxoglutarate \rightarrow small glutamate pool.

One cycle is therefore continuously losing intermediates to the other. This may be balanced by CO_2 fixation in one cycle, forming oxaloacetate from pyruvate, and the reverse reaction in the other cycle, or by a net counter-flow in the opposite direction to γ -aminobutyrate flow, which may involve more than one substance (Garfinkel, 1970).

It has been established that the specific radioactivity of glutamine in mouse brain injected with $[1\text{-}^{14}\text{C}]\text{-acetate}$ reaches a maximum before glutamate (Table 4) followed by a rapid decline in glutamine specific radioactivity. This may be due either to glutamine efflux from the brain (which has never been demonstrated as occurring to a sufficient extent), or to the conversion of glutamine (or some substance equilibrated with it) to glutamate, which may be in either compartment. The $[2\text{-}^{14}\text{C}]\text{-acetate}/[1\text{-}^{14}\text{C}]\text{-acetate}$ labelling ratio increases continuously with time for glutamate, approximating one for the first 20 min (Table 4). If glutamine and its small compartment glutamate precursor equilibrate

rapidly, and if label is lost by the same pathway as it enters, the acetate labelling ratio should be very similar for glutamate and glutamine, contrary to observation. The glutamate from which glutamine is formed therefore differs at least somewhat from that formed from glutamine on the basis of the acetate experiments.

As was previously mentioned, the [2-¹⁴C]acetate/[1-¹⁴C]acetate labelling ratio for glutamine approximates one for 15–20 min, but increases rapidly for glutamate. A [2-¹⁴C]acetate/[1-¹⁴C]acetate labelling ratio of one for glutamine, glutamate or α -oxoglutarate means that most of the radioactivity from [1-¹⁴C]acetate is still in C-5 and most of the radioactivity from [2-¹⁴C]acetate is still in C-4 of these five-carbon compounds (for a more detailed analysis see Van den Berg *et al.* 1969). Indeed the percentage of radioactivity in C-1 of glutamine in experiments with [1-¹⁴C]acetate is smaller than for glutamate. In going from the first to the second turn of the cycle after the entrance of [1-¹⁴C]acetyl-CoA the radioactivity moves from C-5 in α -oxoglutarate to C-1. A small amount of radioactivity in C-1 of glutamine (and therefore very likely in the α -oxoglutarate and glutamate precursor pools of glutamine) indicates appreciable glutamine formation from α -oxoglutarate that leaves the cycle before the second turn. Close matching to the [¹⁴C]acetate data was obtained when approximately 50% of the α -oxoglutarate left the cycle. Sufficiently different labelling of glutamate between [1-¹⁴C]acetate and [2-¹⁴C]acetate was obtained by allowing glutamine (or an equilibrated substance) to move to the large compartment and be converted to glutamate there. The distribution of glutaminase between mitochondrial fractions is consistent with this, as noted above.

Such a flow from the small to the large compartment can be independently determined from the data of Berl *et al.* (1961): the two compartments equilibrate (starting with labelling in the small one), but require more than an hour to do so in the rat. Analysis of their data indicates that aspartate also participates in this flow, although glutamine predominates (Garfinkel, 1970).

A crucial assumption of this model is then that a compound (glutamine) moves from one brain compartment to another and that another compound (γ -aminobutyrate) moves in the opposite direction.

Losses or gains in concentrations of tricarboxylic acid cycle intermediates could be compensated for by CO₂ fixation (or release) which is well established as occurring in brain (Berl, Takagaki, Clarke & Waelsch, 1962; O'Neal & Koeppe, 1966; Salganicoff & Koeppe, 1968), although difficult to assign to specific compartments. The apparent preferential incorporation of ¹⁴CO₂ in glutamine (Berl *et al.* 1962)

might be because the ¹⁴CO₂ is diluted more strongly in the large than in the small compartment. The almost equal incorporation of radioactivity from [3,4-¹⁴C]glucose in glutamate and glutamine in mouse brain (C. J. Van den Berg, unpublished work) argues in favour of this latter possibility. There is no doubt that more experimental data is required to settle these points, and that it is presently impossible to discard the possibility that CO₂ fixation and release are involved in balancing the concentrations of tricarboxylic acid-cycle intermediates in the brain.

It should be kept in mind that this study has been devoted to one particular facet of the complex metabolism of a complex organ, and that the results may therefore suggest that the situation is much simpler than we know it actually is.

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