Glutamic Acid Metabolism in Brain and Liver during Infusion with Ammonia labelled with Nitrogen-15

Recent results of *in vivo* experiments using glutamic acid labelled with carbon-14 were consistent with the hypothesis that the amidation of the administered amino-acid to glutamine represents a metabolic event occurring in a tissue or cellular compartment[1,2]. Since glutamic acid and glutamine syntheses in the central nervous system are the two known processes by which ammonia enters organic linkage, it appeared of great interest to approach the question of setting into compartments the metabolism of glutamic acid and its derivatives with the aid of ammonia labelled with nitrogen-15.

^{15}N-ammonium acetate was infused into the carotid artery of cats over a period of 8–85 min. with simultaneous electroencephalographic and electrocardiographic tracings. The experiments were terminated by exsanguination of the animal, the blood was collected, brain and liver excised and frozen, and the level of glutamic acid and its metabolic derivatives determined in the tissue samples[2,3] as well as the concentration of nitrogen-15 in the amino or amide groups respectively of these compounds. The results of a typical experiment of 25-min. duration are given in Table 1.

Table 1. Isotopic Concentration in Glutamic Acid and Related Metabolites after Nitrogen-15 labelled Ammonia Infusion into the Cat

	Blood		Brain cortex		Liver	
	μM/gm. tissue	N-15 Atom per cent excess	μM/gm. tissue	N-15 Atom per cent excess	μM/gm. tissue	N-15 Atom per cent excess
Ammonia	2·8	81·9	1·8	65·7	4·6	42·6
Glutamine amide	0·32	21·4	7·8	38·7	0·83	28·7
Glutamine α-amino	0·32	1·8	7·8	8·6	0·83	5·4
Glutamic acid	0·08	3·1	9·3	0·87	1·6	13·6

Nitrogen-15 ammonium acetate (99·6 atom per cent excess) 1 mM/ml. infused into carotid artery of a succinylcholine paralysed 3·3 kgm. cat at a uniform rate for 24 min. (total dose, 16 m.moles). Experiment terminated at 25 min. by bleeding from the carotid artery; cortex and liver frozen *in situ* with solid carbon dioxide.

While the concentrations of the amino-acids measured in the various tissues remained constant or decreased slightly, the level of glutamine in the brain increased by at least 50 per cent (for normal values see ref. 4). The observation that the nitrogen-15 content of the amide group of cerebral glutamine is higher than that of liver or blood indicates that this amide is synthesized in the brain. Since the level of glutamine in brain increased considerably, the high specific activity of the amide group is probably not due to an exchange with free ammonia but to a net synthesis of glutamine. Circumstantial evidence for glutamine synthesis in the brain has come from experiments on hepatectomized animals[5] and is consistent with the high synthetase activity in this organ[6,7].

Of particular interest is a comparison of the concentration of nitrogen-15 in the α-amino group of glutamic acid and of glutamine isolated from the brain. Since the α-amino group of glutamine has ten times the specific activity found in glutamic acid, brain glutamine must be derived from a compartment of glutamic acid which is not in equilibrium with the total tissue content of this acid unless glutamine is synthesized appreciably by a pathway which does not require glutamic acid as the immediate precursor.

In contrast to the observations on brain, the α-amino group of liver glutamine is of lower specific activity than that of liver glutamic acid, a result which might be expected if the glutamic acid used for glutamine synthesis in the liver mixes rapidly with the total tissue pool of this amino-acid. However, in preliminary double-labelling experiments (^{14}C glutamic acid and $^{15}NH_3$) compartmentalization of glutamic acid used for glutamine synthesis can also be demonstrated in this organ in the presence of high ammonia concentrations.

If glutamic acid is utilized for the synthesis of the additional glutamine in the brain, the former amino-acid is apparently made in this organ and not derived from the blood, since the nitrogen-15 content of the α-amino group of brain glutamine (8·6 per cent) is higher than that of blood glutamic acid (3·1 per cent).

According to present evidence the reductive amination of keto-glutaric acid occurs in the mitochondria[8] while glutamine synthetase is localized in microsomes and mitochondria[9]. Further experiments will have to show whether the results of this and previous work together with evidence for the enzyme distribution in the cell are indicative of different metabolic pools within the cells for the metabolism of administered glutamic acid and of that synthesized in the tissue. This type of investigation may point the way by which the problem of setting into compartments of metabolic events *in vivo* may be approached[10]. A detailed account of the experiments as well as of the effects of ammonia infusion will be given elsewhere.

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Alpha-Activity of Wheat and Flour

In their work on naturally occurring radioactivity in man and his environment, Mayneord and his co-workers have indicated[1,2] the wide range of alpha-radioactivity found in the more common foodstuffs. Using the same experimental method, we have recently made measurements on a number of samples of wheat and wheat products of known origin, and