Conversion of [U-¹⁴C]Threonine into ¹⁴C-Labelled Amino Acids in the Brain of Thiamin-Deficient Rats

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In confirmation of the findings of Gaitonde *et al.* (1974), a decrease in the brain concentration of threonine and serine, and an increase in glycine, were observed in rats maintained on a thiamin-deficient diet. Similar changes were found in the blood, and the concentration of several other amino acids in the blood decreased significantly. There was a correlation between the concentrations of threonine, serine, aspartate and asparagine in the brain and blood. In experiments in which [U-¹⁴C]threonine was injected into rats most of the radioactivity in the brain and blood of control rats was, as expected, in threonine in the acid-soluble metabolites. In contrast, a considerable proportion of radioactivity was also found in other amino acids, namely glutamate, glutamine, aspartate, γ -aminobutyrate and alanine, in the brain of thiamin-deficient rats. [U-¹⁴C]Threonine was also converted into ¹⁴C-labelled lactate and glucose, but the extent of this conversion was severalfold higher in thiamin-deficient than in control rats. This finding gave evidence of the stimulation in thiamin-deficient rats of the catabolism of [U-¹⁴C]threonine to [¹⁴C]lactate by the amino-acetone pathway catalysed by threonine dehydrogenase, and into succinate via propionate by the α -oxobutyrate pathway catalysed by threonine dehydratase (deaminase). The measurement of specific radioactivities of glutamate, aspartate and glutamine after injection of [U-¹⁴C]threonine, and of lactate and glucose in the brain after injection of [U-¹⁴C]threonine, indicated a stimulation of the activities of threonine dehydrogenase and threonine dehydratase (deaminase) in the brain of thiamin-deficient rats. The specific radioactivities of glutamate, aspartate and glutamine in the brain were consistent with an alteration in the metabolism of threonine, mainly in the 'large' compartment of the brain of thiamin-deficient rats. The measurement of relative specific radioactivity of proteins after injection of [U-¹⁴C]threonine indicated a marked decrease in the synthesis of proteins, mainly in the liver of thiamin-deficient rats.

The maintenance of rats on a thiamin-deficient diet has been shown to result in a decrease in the concentration of glutamate, threonine and serine, and an increase of glycine, in the brain (Gaitonde *et al.*, 1974). However, an acute deficiency of thiamin produced by injection of pyrithiamin, an antagonist of thiamin, to rats maintained on a thiamin-deficient diet showed more marked changes in the concentration of these and other amino acids of the brain, except that the concentration of threonine showed an increase instead of the expected decrease (Gaitonde *et al.*, 1975). These variable changes in the concentration of threonine, an essential amino acid, in the brain suggested an alteration in the metabolic fate of threonine in thiamin deficiency in rats.

The present investigation was undertaken to follow the metabolic fate of [U-¹⁴C]threonine in control and thiamin-deficient rats.

Experimental

Animals

Black hooded male rats of an inbred strain (white and grey) of the MRC Dunn Nutritional Laboratory,

Cambridge, U.K., were used. Littermates were separated into two groups; one group (the control group) was maintained on a thiamin-supplemented diet and the other group on a thiamin-deficient diet from day 24 after birth. The composition of the diet and other treatments were as described by Gaitonde *et al.* (1974).

The animals were used on day 46 after birth, i.e. after 22 days of maintenance on the special diet. The numbers of animals decapitated for measurement of metabolites in the brain and blood are given in appropriate Tables. The metabolism of threonine was studied by injecting four control and four thiamin-deficient rats subcutaneously with 0.5 ml of a solution containing 5 μ Ci of carrier-free L-[U-¹⁴C]-threonine per 100 g body wt. Labelled threonine (228 μ Ci/ μ mol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. The animals were killed by decapitation at different times after injection. The brain was removed quickly, blotted on Whatman no. 50 paper and frozen in liquid N₂ within 30–35 s after decapitation. Blood was collected from the severed neck into a 10 ml beaker

wetted with heparin. The tissue and blood were treated with ice-cold 5% (w/v) HClO_4 and the acid extract was neutralized with KOH as described (Gaitonde, 1974; Gaitonde *et al.*, 1974). The total volume of the acid extract was calculated by assuming that 80% of the tissue weight or blood volume was water.

Fractionation of metabolites

Fractions containing (i) amino acids, (ii) free sugars, (iii) taurine, (iv) lactate, (v) sugar phosphates, nucleotide monophosphates, *N*-acetylaspartate and organic acids, and (vi) nucleotide triphosphates were obtained by ion-exchange chromatography of the neutralized HClO_4 extract as described (Gaitonde *et al.*, 1974, 1975).

Tissue protein

The tissue residue insoluble in HClO_4 was treated a further six times with 5% (w/v) HClO_4 . After each treatment the suspension was centrifuged for 20 min at 3000 rev./min at between 4° and 6°C and the supernatant solution was decanted and discarded. Lipids were extracted from the tissue residue sequentially by treatment with different organic solvents and the combined lipid extract was tested for radioactivity (Gaitonde & Arnfred, 1971).

The final lipid-free residue contained RNA, DNA and protein; it was dried in air at room temperature (20°C), weighed and designated tissue protein. A sample of the tissue protein was dissolved to give a concentration of 10 mg dry wt./ml of 0.5M-NaOH by treatment at 40°C for 24 h. A portion (1.0 ml) was acidified with 0.2 ml of 98–100% (v/v) formic acid and mixed with 10 ml of a scintillation mixture of toluene and Triton X-100 for measurement of radioactivity (see Gaitonde, 1974). Another portion was diluted with 0.5M-NaOH and analysed for protein content by the Technicon Autoanalyzer method (Gaitonde & Arnfred, 1971). Proteins were hydrolysed by refluxing with 6M-HCl for 24 h.

Analytical methods

γ -Aminobutyrate was isolated with Amberlite CG 50 (H^+ form) ion-exchange resin, glutamate, aspartate and GSSG with AG1 ion-exchange resin (acetate form; supplied by BioRad Laboratories, Richmond, Calif., U.S.A.), and other amino acids with Chromobeads type A ion-exchange resin equilibrated with lithium citrate buffer, pH 3.0. Since the concentrations of individual amino acids in the blood were not very different from each other, unlike those of glutamate, aspartate and glutamine in the brain, the amino acid fraction of the blood was chromatographed directly on a 130 cm-long column of Chromobeads type A ion-exchange resin equilibrated with 0.275M-lithium citrate, pH 2.7. The amino acids were eluted with the same buffer, which gave a resolution of glutamine (up to 0.7 μmol) from

Table 1. Concentration of amino acids in the brain and blood of control and thiamin-deficient rats
Values are means $\pm$ s.e.m. Four animals were used for each determination. n.s., Not significant.

Amino acids	Brain				Blood			
	Control (μmol of amino N/g)	Thiamin-deficient (μmol of amino N/g)	Change (%)	P	Control (μmol of amino N/ml)	Thiamin-deficient (μmol of amino N/ml)	Change (%)	P
Total acid-soluble fraction	32.50 $\pm$ 0.355	31.15 $\pm$ 0.512	-4	n.s.	7.12 $\pm$ 0.147	4.92 $\pm$ 0.175	-31	<0.001
Total amino acid fraction*	25.21 $\pm$ 0.686	25.19 $\pm$ 0.427	<1	n.s.	4.84 $\pm$ 0.194	2.71 $\pm$ 0.176	-44	<0.001
Threonine	0.86 $\pm$ 0.003	0.64 $\pm$ 0.027	-26	<0.002	0.49 $\pm$ 0.063	0.23 $\pm$ 0.013	-53	<0.02
Serine	1.07 $\pm$ 0.018	0.88 $\pm$ 0.035	-18	<0.01	0.57 $\pm$ 0.032	0.23 $\pm$ 0.011	-60	<0.001
Glutamate	11.09 $\pm$ 0.287	10.70 $\pm$ 0.191	-4	n.s.	0.48 $\pm$ 0.025	0.32 $\pm$ 0.035	-33	<0.02
Glutamine	4.35 $\pm$ 0.179	4.23 $\pm$ 0.123	-3	n.s.	0.29 $\pm$ 0.017	0.17 $\pm$ 0.021	-41	<0.01
Aspartate	2.35 $\pm$ 0.111	2.08 $\pm$ 0.104	-11	n.s.	0.07 $\pm$ 0.003	0.03 $\pm$ 0.003	-57	<0.001
γ -Aminobutyrate	1.97 $\pm$ 0.005	1.97 $\pm$ 0.007	0	n.s.				
Glycine	0.93 $\pm$ 0.043	1.23 $\pm$ 0.052	+32	<0.01	0.18 $\pm$ 0.016	0.22 $\pm$ 0.016	+22	n.s.
Alanine	0.65 $\pm$ 0.023	0.70 $\pm$ 0.028	+8	n.s.	0.89 $\pm$ 0.012	0.37 $\pm$ 0.045	-58	<0.001
Asparagine ($\times 10$)	0.46 $\pm$ 0.008	0.54 $\pm$ 0.009	+17	n.s.	0.40 $\pm$ 0.001	0.39 $\pm$ 0.004	-2	n.s.
GSSG	0.80 $\pm$ 0.051	0.79 $\pm$ 0.063	-1	n.s.				

* The amino acids in the neutralized HClO_4 -soluble fraction were absorbed on a cation-exchange resin Zerolit 225 and eluted with 1M- NH_3 solution. Taurine, being not absorbed by this resin, was not present in the amino acid fraction.

Table 2. Concentration of glucose, glucose 6-phosphate and lactate in brain and blood of control and thiamin-deficient rats

The brain was removed and frozen in liquid N₂ within 30–35 s after decapitation of the animals. Values are means $\pm$ S.E.M. with the number of determinations in parentheses. n.s., Not significant.

Metabolites	Brain			Blood		
	Control (μ mol/g)	Thiamin-deficient (μ mol/g)	P	Control (μ mol/ml)	Thiamin-deficient (μ mol/ml)	P
Glucose	0.36 $\pm$ 0.01 (20)	0.52 $\pm$ 0.02 (23)	<0.001	6.42 $\pm$ 0.27 (4)	5.52 $\pm$ 0.21 (7)	<0.05
Glucose 6-phosphate	0.07 $\pm$ 0.003 (19)	0.07 $\pm$ 0.004 (24)	n.s.	0.05 $\pm$ 0.003 (4)	0.05 $\pm$ 0.002 (7)	n.s.
Lactate	8.15 $\pm$ 0.22 (16)	8.55 $\pm$ 0.20 (16)	n.s.	1.84 $\pm$ 0.23 (4)	3.53 $\pm$ 0.25 (7)	<0.001

glutamate (up to 1.2 μ mol) in the sample. Further details of methods for the quantitative determination of the concentrations and specific radioactivities of amino acids have been published (Gaitonde *et al.*, 1974; Gaitonde, 1974).

The concentration and specific radioactivity of lactate (Gaitonde *et al.*, 1974), glucose and glucose 6-phosphate (Gaitonde *et al.*, 1975) were determined by enzymic methods.

Results

Concentration of amino acids in brain and blood

The total amino N content of the brain did not change in animals maintained on a thiamin-deficient diet (Table 1). However, as shown (Gaitonde *et al.*, 1974), the concentrations of threonine and serine decreased whereas that of glycine increased significantly in the brain as a result of thiamin deficiency. In contrast, the total amino N content of the blood decreased significantly in thiamin-deficient rats, attributable largely to a decrease in threonine, serine, glutamate, glutamine, aspartate and alanine concentrations (Table 1). The blood glycine concentration showed some increase. A significant correlation between the concentrations of amino acids in brain and blood was found only for threonine ($r = 0.841$, $n = 12$, $P = 0.001$), serine ($r = 0.853$, $n = 12$, $P = 0.001$), aspartate ($r = 0.622$, $n = 11$, $P = 0.05$) and asparagine ($r = 0.573$, $n = 12$, $P = 0.05$).

The individual amino acid concentrations in the brain of control rats used in the present investigation were lower than those reported by Gaitonde *et al.* (1974). In particular, threonine concentration in the brain of control rats used in the present work (0.86 μ mol/g; Table 1) was considerably lower than that reported (1.09 μ mol/g) by Gaitonde *et al.* (1974). Also three out of 17 control brains (including the four used in the present work) gave threonine concentrations similar to those found in the brain of thiamin-deficient rats.

Concentration of glucose, glucose 6-phosphate and lactate

The blood lactate content of the thiamin-deficient rats was significantly higher and glucose content was

correspondingly lower than in the control rats. As the animals were decapitated, glucose content (0.36 μ mol/g; Table 2) of the brain of control rats was considerably lower than that (1.38 μ mol/g) reported by Veech & Hawkins (1974), who obtained samples of brain from the animals by the freeze-blowing technique, which is claimed to give values of glycolytic intermediates very near to those *in vivo*. Despite the limitation of partial post-mortem glycolysis of glucose into lactate, a significant increase in brain glucose content was found in thiamin-deficient rats. This finding agrees with the results of experiments in which a deficiency of thiamin was produced by treatment of rats with pyriethiamin, an antagonist of thiamin (Holowach *et al.*, 1968; Gaitonde *et al.*, 1975).

A deficiency of thiamin, whether produced by feeding a thiamin-deficient diet alone as reported in the present work (Table 2) or by administering pyriethiamin as reported by Gaitonde *et al.* (1975), showed no changes in the concentration of glucose 6-phosphate in brain and blood.

Incorporation of ¹⁴C in brain and blood constituents after injection of [U-¹⁴C]threonine

Information on the metabolic fate of injected [U-¹⁴C]threonine was obtained by measurement of the radioactivity of fractions containing acid-soluble metabolites, lipids or proteins. Radioactivity was found in acid-soluble metabolites and proteins; the lipid fraction contained no measurable amount of radioactivity.

Labelling of proteins

Of the total ¹⁴C content in blood, proteins accounted for 1% at 10 min and 30% at 30 min in the control rats; in contrast there was no detectable radioactivity at 10 min and 7% at 30 min in the thiamin-deficient rats (Table 3). Proteins in the brain, unlike those in blood, were labelled at 10 min after injection of [U-¹⁴C]threonine into thiamin-deficient as well as control rats. The percentage incorporation of ¹⁴C into the brain proteins at 10 min was similar in both control and thiamin-deficient rats, but it was considerably less at 30 min in thiamin-deficient (4%) than in the control (13%) rats (Table 3).

Table 3. ^{14}C content of protein and acid-soluble metabolites of the brain and blood of control and thiamin-deficient rats after injection of $[\text{U-}^{14}\text{C}]\text{threonine}$

The animals were killed by decapitation at 10 and 30 min after injection of $5\text{ }\mu\text{Ci}$ of $[\text{U-}^{14}\text{C}]\text{threonine}$. The radioactivity in the free sugar fraction of the brain was all due to $[\text{U-}^{14}\text{C}]\text{glucose}$. However, $[\text{U-}^{14}\text{C}]\text{glucose}$ accounted for 20–40% and 80% of the radioactivity in the free sugar fractions of blood of control and thiamin-deficient rats respectively. The values given in the Table are the mean of two determinations except that for the blood of the thiamin-deficient rat at 10 min, which is from one animal only. The duplicates were within $\pm 20\%$ of the mean.

Tissue constituents	Time after injection	Radioactivity in brain (d.p.m./g)				Radioactivity blood (d.p.m./ml)			
		10 min		30 min		10 min		30 min	
		Control	Thiamin-deficient	Control	Thiamin-deficient	Control	Thiamin-deficient	Control	Thiamin-deficient
Protein		300	300	2140	1370	850	*	13430	3620
HClO_4 extract		9230	9950	14840	32000	71000	59570	31190	47780
Amino acid fraction		8130	5460	13080	19870	59180	41840	27160	17350
Free sugar fraction		*	330	*	1190	110	2140	140	6650
Lactate		210	2900	410	7220	150	5640	180	17460
Sugar phosphate fraction		120	410	190	920	50	60	40	480
Nucleoside triphosphate fraction		60	120	140	270	12	*	15	390
Taurine fraction		280	160	440	230	1010	2220	1110	440
Recovery from fractions of HClO_4 extract (%)		95.3	94.3	96.1	92.8	85.2	87.1	91.8	89.5

* Amount of radioactivity not measurable.

Table 4. Incorporation of $[\text{U-}^{14}\text{C}]\text{threonine}$ into proteins of control and thiamin-deficient rats

The relative specific radioactivity is given by $\frac{10^5 \times \text{d.p.m./mg of protein}}{\text{d.p.m./}\mu\text{mol of threonine in HClO}_4 \text{ extract}}$. The values given in the Table are the mean of duplicate determinations, which were within $\pm 20\%$ of the mean.

Index of incorporation	Time after injection	Brain				Blood			
		10 min		30 min		10 min		30 min	
		Control	Thiamin-deficient	Control	Thiamin-deficient	Control	Thiamin-deficient	Control	Thiamin-deficient
$10^{-3} \times$ Specific radioactivity of threonine (d.p.m./ μmol)		9.4	10.0	18.0	16.4	124.8	199.1	61.7	110.3
Specific radioactivity of protein (d.p.m./mg)		3.1	3.1	21.7	13.8	5.4	*	78.7	22.7
Relative specific radioactivity of protein		33	31	120	84	4	*	127	20

* Amount of radioactivity not measurable.

Ion-exchange chromatography of protein hydrolysates showed that the radioactivity in proteins from brain and blood was essentially due to incorporation of threonine, except that a small amount of radioactive glycine (4–6 d.p.m./mg of protein) was also detectable in the blood proteins of the control rats 30 min after injection of [U- ^{14}C]threonine. Other amino acids in proteins from the brain and blood contained a negligible amount of radioactivity (less than 25% of the background counting rate) in both the control and thiamin-deficient rats. These findings show that ^{14}C -labelled glutamate, glutamine, aspartate and alanine found in the HClO_4 extract of the brain of thiamin-deficient rats after injection of [U- ^{14}C]threonine (see below) were not readily available for their incorporation into the proteins of brain.

The specific radioactivity of proteins, expressed as d.p.m./mg of protein of brain at 10 min, was similar in both control and thiamin-deficient rats, but at 30 min the specific radioactivity of the protein of brain as well as blood was considerably less in thiamin-deficient rats than in control rats (Table 4). The same conclusion may be drawn from measurement of relative specific radioactivity of proteins, an index of incorporation that corrects for differences in the specific radioactivity of precursor [U- ^{14}C]threonine in the HClO_4 extract (Table 4). Since liver is the main source of blood proteins, the observed marked decrease in the specific radioactivity and relative specific radioactivity of blood proteins indicates that the incorporation of [U- ^{14}C]threonine into liver proteins decreased considerably in thiamin-deficient rats.

Labelling of acid-soluble metabolites

There was a decrease in ^{14}C in the acid-soluble metabolites of blood with time after injection of [U- ^{14}C]threonine: in the control rats the value at

30 min was approx. 44% of that at 10 min, but it was approx. 80% in the thiamin-deficient rats (Table 3). Although the concentration of ^{14}C was considerably lower in the brain than in blood at 10 and 30 min after the injection of [U- ^{14}C]threonine, the ^{14}C content of the brain increased during this period by approx. 60% in the control and 220% in the thiamin-deficient rats.

As expected, most of the radioactivity in the acid-soluble metabolites of both the brain and blood of control rats was in the amino acid fraction (Table 3). However, inspection of the distribution pattern of ^{14}C in several groups of metabolites revealed a distinct difference in two respects in the metabolic fate of [U- ^{14}C]threonine between the control and thiamin-deficient rats. (i) A significant proportion of the radioactivity in the acid-soluble extract was in lactate and free sugars of the blood and brain of thiamin-deficient rats, and (ii) although small amounts of [^{14}C]lactate and free sugars were found in the blood of control rats, only [^{14}C]lactate was measurable in the brain of control rats.

A considerable proportion of ^{14}C was also found in the taurine fraction, which contains taurine, phosphoethanolamine and glycerophosphoethanolamine. The ^{14}C -labelled compound in this fraction has not been identified. A small but measurable amount of radioactivity was also present in the sugar phosphate and nucleoside triphosphate fractions (Table 3).

^{14}C content of individual amino acids. Threonine accounted for 95–98% of the radioactivity of amino acids in the brain and blood of control rats after the injection of [U- ^{14}C]threonine. Of the several amino acids examined only glutamate (0.5–1.0%) in brain, and glycine (0.05–0.5%) and serine (0.3–0.4%) in blood, were found labelled at 10 and 30 min. In view of the isotopic resolution of ^{12}C -labelled and ^{14}C -

Table 5. Incorporation of ^{14}C into amino acids of brain and blood of thiamin-deficient rats after injection of [U- ^{14}C]threonine

The radioactivity in the amino acid fraction of control rat brain and blood was essentially in threonine (see the text). The values given are means of duplicate determinations which were within $\pm 5\%$ of the mean.

	Time after injection ...	^{14}C in the amino acid fraction (%)			
		Brain		Blood	
		10min	30min	10min	30min
Threonine		82.3	46.1	95.9	81.1
Serine		0.6	0.1	0.4	0.4
Glutamate		10.0	25.1	*	*
Glutamine		3.1	10.9	*	*
Aspartate		3.0	8.2	*	*
γ -Aminobutyrate		1.3	3.2		
Alanine		0.6	0.9	*	*
Glycine		*	*	0.3	0.2
GSSG		0.8	1.3		

* Amount of radioactivity not measurable.

labelled amino acids on ion-exchange resin columns (Gaitonde & Nixey, 1972), the presence of a small amount of radioactivity in serine is probably due to overlapping traces of [^{14}C]threonine.

In contrast, a considerable proportion of radioactivity in amino acids of the brain after injection of [$\text{U-}^{14}\text{C}$]threonine into thiamin-deficient rats was found also in other amino acids, namely glutamate, glutamine, aspartate, γ -aminobutyrate, alanine and the tripeptide glutathione (Table 5).

The recovery of ^{14}C after ion-exchange chromatography of the amino acid fraction was between 96 and 102% for both brain and blood, except that the amino acid fraction of blood obtained at 30 min after injection to thiamin-deficient rats only, gave a recovery of 82% of the radioactivity.

Discussion

Whereas essentially all the radioactivity in the acid-soluble fraction of the brain was in threonine in the control rats, a considerable proportion of the radioactivity in this fraction of the brain of thiamin-deficient rats was also in lactate, glucose and amino acids, particularly those closely associated with the tricarboxylic acid cycle. Clearly [$\text{U-}^{14}\text{C}$]threonine injected into thiamin-deficient rats was catabolized, and this raises the question of the pathways of its catabolism and the site of catabolism, i.e. whether the injected [$\text{U-}^{14}\text{C}$]threonine was catabolized in the brain or whether the ^{14}C -labelled catabolic products of [$\text{U-}^{14}\text{C}$]threonine were transported to the brain from other organs by the bloodstream.

Labelled metabolites in blood

The incorporation of ^{14}C into blood glucose and lactate after injection of [$\text{U-}^{14}\text{C}$]threonine was greatly enhanced by thiamin deficiency. The [^{14}C]glucose must originate in the liver and kidney by gluconeogenesis from the [$\text{U-}^{14}\text{C}$]threonine, most probably via the α -oxobutyrate pathway of threonine catabolism. For the enzymes governing the three catabolic pathways of threonine (see Scheme 1) the highest activities reported have been with the liver dehydratase and methylmalonyl-CoA mutase, enzymes of the α -oxobutyrate pathway (Greenberg, 1961; Goldstein *et al.*, 1962). It is also noteworthy that the amount of [^{14}C]glucose was 5–15-fold that of [^{14}C]lactate, and that aspartate, glutamate, glutamine and alanine were labelled in the blood at 10 and 30 min after subcutaneous injection of [$2\text{-}^{14}\text{C}$]propionate (an intermediate on the α -oxobutyrate pathway) in control rats (M. K. Gaitonde, unpublished work). However, the ^{14}C content of blood lactate after injection of [$\text{U-}^{14}\text{C}$]threonine was similar to that of glucose in control rats and 2–3-fold that of glucose in thiamin-deficient rats (Table 3); the specific radioactivity of lactate was 3–8-fold that of glucose

Table 6. Specific radioactivities (d.p.m./ μmol) of glucose, glucose 6-phosphate and lactate in brain and blood of control and thiamin-deficient rats after injection of [$\text{U-}^{14}\text{C}$]threonine

Time after injection ...	Brain				Blood			
	10 min		30 min		10 min		30 min	
	Control	Thiamin-deficient	Control	Thiamin-deficient	Control	Thiamin-deficient	Control	Thiamin-deficient
Glucose	*	340	*	780	10	480	5	770
Glucose 6-phosphate	*	350	*	660	*	*	*	*
Lactate	25	350	40	965	80	1350	105	4520

* Amount of radioactivity not measurable.

Table 7. Specific radioactivities of amino acids in the brain of thiamin-deficient rats after injection of [U-¹⁴C]threonine

Only glutamate contained a measurable amount of radioactivity (specific radioactivity 5 at 10min and 11 at 30min) in the brain of control rats. The duplicate determinations at 10min were within ±10% of the mean value given.

Time after injection ...	Sp. radioactivity (d.p.m./μmol)		
	10min	30min	
		Expt. (i)	Expt. (ii)
Glutamate	70	680	320
Glutamine	50	600	280
Aspartate	140	950	470
γ-Aminobutyrate	50	490	200
Alanine	60	270	180

in blood and similar in brain (Table 6). Moreover, since aspartate, alanine, glutamate and glutamine were not labelled in measurable amounts in the blood of thiamin-deficient rats (Table 5), [¹⁴C]lactate formed via pyruvate by decarboxylation of oxaloacetate derived by the α-oxobutyrate pathway in these animals must be considered negligible. It is concluded that the greater proportion of [¹⁴C]lactate was derived from [U-¹⁴C]threonine by the aminoacetone pathway, as originally proposed for the catabolism of threonine (and utilization of glycine) in bacterial cells and animal tissues (Elliott, 1959; Green & Elliott, 1964). Taking account of all observations it would appear that both threonine dehydratase and threonine dehydrogenase activities are stimulated in thiamin deficiency.

The main source of [¹⁴C]lactate and [¹⁴C]glycine in blood must be liver and kidney, where [U-¹⁴C]threonine may be converted into amino[¹⁴C]acetone by threonine dehydrogenase (Green & Elliott, 1964), or by aminoacetone synthetase (Urata & Granick, 1963) after the conversion of [U-¹⁴C]threonine into [¹⁴C]glycine by threonine aldolase (Braunshtein & Vilenkina, 1949; Lin & Greenberg, 1954; Karasek & Greenberg, 1957; Malkin & Greenberg, 1964; Riario-Sforza *et al.*, 1969). Aminoacetone is a normal constituent of urine and its excretion in urine is increased on administration of threonine to rats (Marver *et al.*, 1966). Direct conversion of [U-¹⁴C]threonine into amino[¹⁴C]acetone in blood is ruled out in view of the absence of threonine dehydrogenase from rat blood (Green & Elliott, 1964). Although reported present in duck erythrocytes (Meltzer & Sprinson, 1952) and chicken erythrocytes (Gibson *et al.*, 1958), threonine aldolase and aminoacetone synthetase also are probably not present in rat blood and this would exclude the possibility of synthesis of amino[¹⁴C]acetone in blood from [U-¹⁴C]threonine. Further oxidation of amino[¹⁴C]acetone to [¹⁴C]lactate must have occurred in rat liver and kidney in view of the presence in these tissues of amine oxidase (Urata &

Granick, 1963), aminoacetone-2-oxoglutarate aminotransferase (Urata & Granick, 1963), methylglyoxalase (Dakin & Dudley, 1913; Ariyama, 1928; Dickens, 1933; Jowett & Quastel, 1934; Platt & Schroeder, 1934; Hopkins & Morgan, 1948) and D-lactate-cytochrome *c* reductase (Tubbs & Greville, 1961). L-[¹⁴C]Lactate, finally formed from [¹⁴C]pyruvate in these tissues by L-lactate dehydrogenase, would be transported into the blood. Alternatively, a part of the amino[¹⁴C]acetone formed in the tissues was transported to the blood, where it was converted into [¹⁴C]lactate via [¹⁴C]methylglyoxal by the catalytic action of amine oxidase (Elliott, 1960; Buffoni & Blaschko, 1963) and methylglyoxalase (Dakin & Dudley, 1913; Ariyama, 1928; Jowett & Quastel, 1933). Brain may also be considered a source of blood-borne [¹⁴C]lactate (see below).

The aldolase activity, with the formation of glycine, accounts for 20–33 % of threonine catabolized in normal animals (Meltzer & Sprinson, 1952). In the present investigation the evidence for the activity of this enzyme was obtained in all control rats and only in some thiamin-deficient rats. The absence of [¹⁴C]glycine from the brain agrees with the reported absence of threonine aldolase from rat brain (Braunshtein & Vilenkina, 1949) and indicates that brain cannot be a source of [¹⁴C]glycine in the blood.

Labelled metabolites in brain

The conversion of threonine into α-oxobutyrate was demonstrated in rat brain preparations (Salvatore *et al.*, 1966) and α-oxobutyrate was oxidatively decarboxylated to propionate by pigeon brain preparations (Long, 1943). Further oxidation of propionate to succinate was catalysed by mitochondrial enzymes in mammalian brain (Beck *et al.*, 1957). In view of the absence of any measurable amount of radioactivity in aspartate in the brain of control rats at 10 and 30min after injection of [U-¹⁴C]threonine, catabolism of threonine by the α-oxobutyrate path-

way in the brain of control rats must be considered negligible. If this pathway was operating in the brain, aspartate would be the first amino acid to be labelled from [U- 14 C]threonine in the brain and its specific radioactivity would be expected to be higher than that of glutamate and glutamine. This was indeed the case in the brain of thiamin-deficient rats (Table 7). The higher labelling of aspartate in the brain could not have been due to the entry of 14 C-labelled propionate (and presumably 14 C-labelled α -oxobutyrate) formed by catabolism of [U- 14 C]threonine in liver, because intraperitoneally administered [14 C]propionate has been reported to give the specific radioactivity of aspartate considerably less than that of glutamate (approx. 61–73%; O'Neal *et al.*, 1966). Also the 14 C content of aspartate was approx. 35% of that of glutamate at 30 min after injection of [U- 14 C]threonine (Table 5); if the labelling of aspartate was exclusively by metabolism of blood-borne [14 C]glucose in the brain the 14 C content of aspartate would be expected to be approx. 20% (Gaitonde *et al.*, 1974). It is therefore concluded that, unlike in the control rats, a part of the injected [U- 14 C]threonine is catabolized by the α -oxobutyrate pathway to aspartate in the brain of thiamin-deficient rats. In the light of the present evidence in favour of increased threonine dehydratase activity in the brain of thiamin-deficient rats, it is suggested that the reported variable concentrations of threonine dehydratase in liver (Nishimura & Greenberg, 1961; Doonan *et al.*, 1974) may be related to thiamin concentrations in animal liver.

Since radioactive glucose was not present in measurable amounts in the brain of control rats after injection of [U- 14 C]threonine, it could not have been the source of labelled lactate in their brain. The evidence for the entry of blood-borne [14 C]lactate into the brain was obtained by O'Neal & Koeppe (1966) for adult rats and Cremer & Heath (1974) for 17-day-old suckling rats. A calculation based on the data of Cremer & Heath (1974) for the amount and specific radioactivity of lactate in brain after injection of [14 C]lactate shows that [14 C]lactate content (d.p.m./g) in the brain of 17-day-old suckling rats was approx. 30% of that in the blood (d.p.m./ml). It may be assumed that this value is likely to be considerably less in the older rats, e.g. in the 46-day-old rats used in the present investigation, owing to the operation of the blood-brain barrier. The 14 C content of brain lactate found in the present work after injection of [U- 14 C]threonine was approx. $189 \pm 32\%$ (S.E.M.) of that in the blood of control rats and therefore indicates that it cannot be due to trapped blood in the brain and that not all [14 C]lactate found in the brain could have been due to the entry of blood-borne [14 C]lactate. Therefore it is suggested that [14 C]lactate in the brain of control rats was derived partly by direct entry *in vivo* of blood-borne [14 C]lactate and partly by catabolism of [U- 14 C]threonine in the brain

by the aminoacetone pathway. Moreover, in thiamin-deficient rats, a part of brain [14 C]lactate must also be due to glycolysis, *in vivo* and *post mortem*, of blood-borne [14 C]glucose in the brain.

The conclusion that a part of the [U- 14 C]threonine in the brain was converted into lactate by the aminoacetone pathway agrees with the reported presence in the brain of threonine dehydrogenase, a mitochondrial enzyme (Green & Elliott, 1964), amine oxidase, also a mitochondrial enzyme (Urata & Granick, 1963) and methylglyoxalase (Dakin & Dudley, 1913; Jowett & Quastel, 1934; Ariyama, 1928), although evidence is lacking for the occurrence of D-lactate-cytochrome *c* reductase.

Site of catabolism of [U- 14 C]threonine in the brain

The results of previous investigations on the labelling of glutamate, glutamine, aspartate and γ -aminobutyrate after injection of different 14 C-labelled precursors have given evidence for the concept of a 'small' and a 'large' glutamate compartment in the brain. The labelling of amino acids is presumed to occur in the 'small' compartment if the observed specific radioactivity of glutamine is greater than that of glutamate, and in the 'large' compartment if otherwise. [For references on this subject see reviews by Balázs *et al.* (1973), Berl (1973), Cremer (1973) and Van den Berg (1973).]

In the present investigation the specific radioactivity of aspartate was higher than that of glutamate after injection of [U- 14 C]threonine. This finding is similar to that to be expected after injection of [14 C]succinate (Möhler *et al.*, 1974). However, the fact that the specific radioactivity of glutamine was not greater than that of glutamate in the present investigation (Table 7), unlike that in experiments after injection of 14 C-labelled succinate (Möhler *et al.*, 1974), propionate (O'Neal *et al.*, 1966) or leucine (Patel & Balázs, 1970), suggests that the labelling of amino acids from [U- 14 C]threonine must have occurred mainly in the 'large' compartment, as in experiments after injection of 14 C-labelled glucose (Cremer, 1964; Gaitonde *et al.*, 1965) and lactate (O'Neal & Koeppe, 1966). Provided that the 'large' compartment is part of the neuronal elements (Balázs *et al.*, 1973), it is suggested that the metabolism of threonine, an essential amino acid, in the neuronal compartment is altered in thiamin deficiency in the rat so that some threonine is catabolized for the synthesis of aspartate, glutamate, glutamine and γ -aminobutyrate by the aminoacetone and α -oxobutyrate pathways as shown in Scheme 1. In view of the multiplicity of the precursors such as 14 C-labelled lactate, succinate and glucose formed by the catabolism of [U- 14 C]threonine, the results of the present investigation do not provide estimates of the relative contributions of labelling of amino acids by these precursors.

Scheme 1. Catabolic pathways of threonine in rat tissues

The presence of aminopropanol dehydrogenase in rat tissues was reported by Turner & Willeits (1967). The evidence for other pathways is quoted in the text. TPP, Thiamin pyrophosphate.

Stimulation of enzymes and amino acid concentrations in the brain

The relevance of a deficiency of thiamin to the apparent stimulation of threonine dehydrogenase and threonine dehydratase is not obvious from the catabolic pathways of threonine shown in Scheme 1. Threonine dehydratase of rat liver is shown to be inducible under a variety of conditions, such as starvation (Pitot *et al.*, 1958), administration of threonine or proteins (Sayre *et al.*, 1956; Goldstein *et al.*, 1962; Pitot & Peraino, 1964; Yamashita *et al.*, 1968), glucocorticoids (Peraino *et al.*, 1966; Peraino, 1967; Ku *et al.*, 1969) and experimentally induced diabetes (Ishikawa *et al.*, 1965). It therefore appears likely that partial starvation in the thiamin-deficient rats was responsible for the apparent enhanced activity of threonine dehydratase and perhaps that of threonine dehydrogenase in the rat. If so, the results of the present investigation suggest that the activities of these two enzymes are induced in the brain, as well as in other organs, by dietary factors such as those imposed by a deficiency of thiamin in the diet. Since the conversion of [U-¹⁴C]glucose into brain amino acids was decreased in thiamin-deficient rats (Gaitonde *et al.*, 1974), the stimulation of threonine dehydratase and threonine dehydrogenase in the brain of thiamin-deficient rats would enable the brain to utilize threonine, an essential amino acid, as an alternative source of precursor for the synthesis of aspartate, glutamate, glutamine and γ -aminobutyrate.

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