Entry of Glucose Carbon into Amino Acids of Rat Brain and Liver *in vivo* after Injection of Uniformly ^{14}C-Labelled Glucose

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1. Measurements were made of the rate of incorporation of ^{14}C from uniformly ^{14}C-labelled glucose into individual amino acids of rat brain and liver. 2. At 2·5 min. after intravenous injection of uniformly ^{14}C-labelled glucose, about 30% of the total radioactivity in the brain was present in the five amino acids studied. At 30 min. after subcutaneous injection the distribution of ^{14}C in amino acids was: in brain, alanine 2%, γ-aminobutyrate 4%, aspartate 9%, glutamine 9% and glutamate 37% (total 69%); in liver, alanine 3%, aspartate 2·6%, glutamine 5·3% and glutamate 5·2% (total 18%). About 1% of the total radioactivity was in serine and glycine. 3. In both organs the specific radioactivity of alanine was initially higher than that of the other amino acids examined. The specific radioactivity of γ-aminobutyrate in the brain was about the same as or higher than that of glutamate. 4. Amino acids of the rat brain were separated into 'free' and 'bound' fractions from brain dispersions in saline (or sucrose) media. Definite differences in the specific activities of the 'bound' and 'free' forms were not apparent.

It has long been generally accepted that glucose is the main source of the carbon atoms utilized in the energy metabolism of the brain. More recently it has become apparent that, though a part of the glucose carbon is metabolized directly to carbon dioxide, a part is retained in the amino acids, proteins and lipids of the brain (Allweis & Magnes, 1958*a*,*b*; Geiger, 1958; Sacks, 1957; Roberts, Flexner & Flexner, 1959; Busch, Fujiwara & Keer, 1960; Vrba, Gaitonde & Richter, 1962; Cremer, 1964). Within 20 min. after subcutaneous injection of uniformly ^{14}C-labelled glucose in the rat or the cat, about 65–72% of the total radioactivity in the brain is found in the amino acids. In contrast the amino acids in tissues such as blood, liver, spleen, lung and kidney accounted for only 2–16% of the total radioactivity (Vrba *et al.* 1962; Gaitonde, Marchi & Richter, 1964).

In the present study, the time-course of the entry of glucose carbon into individual amino acids after subcutaneous and intravenous injection of uniformly ^{14}C-labelled glucose was followed in the rat brain and liver. It is known that γ-aminobutyrate, measured chemically or by its factor I activity (Lovell & Elliott, 1963), and related amino acids (R. A. T. Khan & K. A. C. Elliott, unpublished work) in the brain are present partly in 'free' and partly in 'bound' forms. To test whether these different forms could be distinguished metabolically *in vivo* after intravenous injection of uniformly ^{14}C-labelled glucose the brain was dispersed in physiological saline or sucrose medium, and on centrifugation the 'free' amino acids were obtained in the supernatant fluid and 'bound' amino acids in the sediment. Preliminary reports of this work have been published (Gaitonde, 1964; Elliott, Dahl & Balázs, 1964).

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EXPERIMENTAL

In the first series of experiments (Expts. 1 and 2) male rats (100–110 g.) were injected subcutaneously with 0·5 ml. of uniformly ^{14}C-labelled ([U-^{14}C])glucose soln. containing 10 μc with 1 mg. of carrier glucose. The animals were killed by decapitation, the whole brain and liver were quickly removed, blotted on filter paper and dropped into liquid N_2. (The brain was frozen in less than 1 min. after death.) The tissues from two animals were pooled for measurements at

each time. The tissues were dispersed in ice-cold 6% (w/v) perchloric acid (5 ml./g.) with an electric blender (Ultra-turrax; Janke und Kunkel K.G.), centrifuged in a refrigerated centrifuge at 2000 *g* for 30 min. and the supernatant solution was filtered. The tissue residue was washed once with 5 ml. of perchloric acid and the washing combined with the main extract. In the second series of experiments rats weighing 175 g. were injected intravenously through a tail vein with 0·4 ml. of 0·9% NaCl soln. containing 10 or 20 μc of [U-^{14}C]glucose without added carrier glucose. After decapitation the cerebral hemispheres were removed and suspended at room temperature in 9 vol. of phosphate-buffered (pH 7·6) saline solution resembling cerebrospinal fluid or in ice-cold 0·3 M-sucrose solution, by using the homogenizer described by Aldridge, Emery & Street (1960). [The saline–phosphate medium contained: 102 ml. of NaCl (0·154 M); 3 ml. of KCl (0·154 M); 1 ml. of $MgSO_4$ (0·154 M); 20 ml. of $Na_2HPO_4 + NaH_2PO_4$ (0·11 M; 96:4 v/v).] R. A. T. Khan & K. A. C. Elliott (unpublished work) found that the proportion of γ-aminobutyrate in the particle-bound form in saline suspensions was higher if homogenization was done at room temperature than in the cold; temperature had no such effect on suspension in sucrose medium. The tissue suspension was centrifuged at 22000 *g* for 15 min. at 0°. The supernatant fluid containing 'free' amino acids and the resuspended sediment containing 'bound' amino acids were deproteinized with ice-cold 60% (w/v) perchloric acid. The acid extracts were neutralized to phenolphthalein with 2 N-KOH, kept for 24 hr. and filtered in the cold room. The precipitate of potassium perchlorate was washed once with ice-cold water; the filtrate and the wash fluid were made to volume.

In a typical experiment, 20 ml. (1–2 g. of tissue) of the neutralized perchloric acid extract was passed through a resin column of Zeo-Karb 225 (H^+ form). The absorbed amino acids were eluted from the column with aq. N-NH_3 soln. The eluate was evaporated to dryness at 50–55° under reduced pressure. The residue was dissolved in water and the neutral and dicarboxylic amino acids were absorbed on a column of Dowex 1 (CO_3^{2-} form), eluted with 0·1 N-HCl and collected separately in the initial neutral effluent and the following acid effluent as described by Gaitonde *et al.* (1964).

The neutral and dicarboxylic amino acid fractions were evaporated to dryness at 50–55° under reduced pressure, dissolved in water (0·5 ml./g. of tissue) and analysed for individual amino acids after separation by paper chromatography.

Isolation of amino acids by paper chromatography. Duplicate samples (0·1 ml.) of the neutral amino acid fraction were chromatographed in the descending direction on Whatman no. 1 paper with butan-1-ol–propionic acid–water (10:5:7, by vol.) for 30–34 hr. The chromatogram was dried and cut into two strips; one strip was sprayed with 0·5% ninhydrin soln. (or dipped in 0·25% ninhydrin in acetone) and heated to localize glutamine, alanine and γ-aminobutyrate. In the one-dimensional chromatography with the above solvent system serine and glycine somewhat overlapped the glutamine spot. The neutral amino acid fractions of brain in Expt. 2 and of liver in Expt. 1 were chromatographed further in the second direction with 85% phenol (in the presence of NH_3 vapour) to separate glutamine, serine and glycine. One-dimensional paper chromatography on Whatman no. 1 with isobutanol–formic acid (90%)–water (15:3:2, by vol.) (see Hausmann, 1952) for 40 hr. was also used to separate neutral amino acids of liver (Expt. 2). This solvent system gave good resolution of glutamine, glycine, serine, threonine and alanine; it was not satisfactory for neutral amino acids of brain since in this solvent system γ-aminobutyrate overlapped the alanine spot.

Glutamate and aspartate present in the dicarboxylic amino acid fraction of brain were similarly localized on descending chromatograms developed with 80% phenol on Whatman no. 4 paper for 16–20 hr.

The dicarboxylic amino acid fractions of brain and liver also contained another unidentified amino acid which travelled as a streak partially overlapping aspartate in the phenol system; its amino N content (approx. 0·64 μmole/g. of tissue) was 25% of that of aspartate in brain and 64% that of aspartate in liver. Therefore the amino acids in the dicarboxylic amino acid fraction of liver were separated by one-dimensional chromatography on Whatman no. 1 paper with butan-1-ol–propionic acid–water (10:5:4, by vol.) for 36–40 hr.

After localization of the amino acids they were eluted from the unstained strip in 6 ml. of water. A portion of the eluate (1·5 ml.) was transferred to a planchet for measurement of radioactivity and another portion (3·5–4 ml.) was used for the determination of amino N. Mixtures of known amounts of appropriate amino acids were chromatographed and the fractions eluted and determined under the above conditions.

Since the specific radioactivity of glutamine was considerably lower than that of glutamate (Tables 2 and 4) during the earlier times after injection, any hydrolysis of glutamine into glutamate in the course of the isolation of amino acids might result in lowering the specific radioactivity of glutamate. In an experiment in which glutamine in quantity similar to that present in brain extracts was carried through the entire procedure starting with perchloric acid solution, no more than 2% of the glutamine was decomposed into glutamate. Further, the concentrations of glutamate and glutamine determined by methods described in this work were very similar to those reported in the literature. No correction for glutamine decomposition was therefore applied to the specific radioactivity found for glutamate.

Determination of amino nitrogen. Portions of eluates of amino acids and paper blanks (3·5–4 ml.) were pipetted into 10–15 ml. beakers, made alkaline to phenolphthalein with 0·1 N-NaOH (pH 8·3–8·5) and dried overnight in the presence of NaOH, conc. H_2SO_4 and silica gel *in vacuo* in a desiccator. Losses of amino N, except that of aspartate and glutamate, occurred if desiccation was prolonged more than 24 hr. The residue was dissolved in an appropriate volume of water (2·5–5·0 ml.) to give 1–3 μg. of amino N/ml. Duplicate samples (1 ml.) were analysed for amino N according to the method of Yemm & Cocking (1955). The mean extinction values/μg. of amino N in 5 ml. final volume at 570 mμ in cells with 1 cm. light-path were: glutamate, 0·280 ± 0·010; aspartate, 0·276 ± 0·010; alanine, 0·276 ± 0·010; glutamine, 0·250 ± 0·015; γ-aminobutyrate, 0·204 ± 0·010; serine, 0·276 ± 0·005; glycine, 0·272 ± 0·005.

Measurement of radioactivity. All samples were transferred to a planchet and counted at infinite thinness in a Nuclear–Chicago gas-flow counter with a Micromil window as described by Vrba *et al.* (1962). The results were corrected for background counting rate.

Table 1. *Distribution of* ^{14}C *in amino acids of rat brain after subcutaneous injection of* [*U*-^{14}C]*glucose*

Percentages are with reference to total radioactivity in the extract. Serine and glycine contained measurable amounts of radioactivity (0·4–0·7%) at 30, 60 and 120 min.

Time after injection (min.)	Expt. no.	Counts/min./g. of fresh tissue: Perchloric acid extract	Counts/min./g. of fresh tissue: Amino acids	^{14}C in amino acids (% of total radioactivity)	Percentage distribution of ^{14}C: Alanine	Glutamate	Glutamine	γ-Amino-butyrate	Aspartate	Total recovered
5	1	1430	280	19·6	4·6	11·3	5·1	4·1	2·0	27·3
	2	4200	970	23·0	2·6	12·4	2·2	1·0	2·2	20·5
10	1	6850	1840	26·9	2·7	16·8	4·2	2·0	3·2	29·1
	2	6110	2230	36·5	2·4	21·2	4·7	2·0	4·0	34·4
20	1	7040	3700	52·5	4·2	29·2	9·3	4·2	6·3	53·3
	2	13960	7630	54·7	1·7	27·6	5·0	—	6·6	—
30	1	21540	14090	65·5	3·0	34·3	8·7	4·9	8·1	58·7
	2	24930	18080	72·5	1·6	40·4	9·6	3·0	10·4	65·8
60	1	21900	15060	68·8	1·2	35·3	14·1	4·9	8·0	63·4
	2	18260	13370	73·2	1·2	38·8	15·9	4·1	8·1	69·4
120	1	8170	5140	62·8	1·1	29·4	15·2	4·6	6·2	56·4
	2	13080	10040	76·7	0·7	32·1	16·7	4·0	10·1	65·0

RESULTS

Rate of entry of ^{14}C *into amino acids after subcutaneous injection of* [*U*-^{14}C]*glucose*

Brain. The total radioactivity per g. of fresh tissue at any given time differed in the two experiments, but the proportions of ^{14}C incorporated into the amino acid fraction were similar (Table 1). The labelling of the total amino acid fraction reached the highest value (65–77%) during 30–120 min. after the injection of glucose. About 90–110% of the radioactivity in the amino acid fraction was found in alanine, glutamate, glutamine, aspartate and γ-aminobutyrate; serine and glycine contained only negligible amounts. At 30 min. 37% of the radioactivity in the brain was in glutamate, 9% in glutamine, 9% in aspartate, 4% in γ-aminobutyrate and 2% in alanine (mean values are given in Table 1).

The specific radioactivities of these amino acids are expressed as counts/min./μg.atom of carbon. The results of Expt. 1 are shown graphically in Fig. 1. The average specific radioactivity of alanine was higher than that of other amino acids during the first 30 min., at which time it had reached its peak value; it then decreased rapidly with time. Other amino acids showed the highest specific radioactivity between 30 and 60 min. after injection. At 120 min. the amino acid carbon had almost reached isotopic equilibrium. The observed specific radioactivity of glutamate was at all times proportional to the total radioactivity in the perchloric acid extract or the amino acid fraction of the brain (Fig. 2).

The labelling pattern of γ-aminobutyrate indicates that labelled glutamate in brain was rapidly

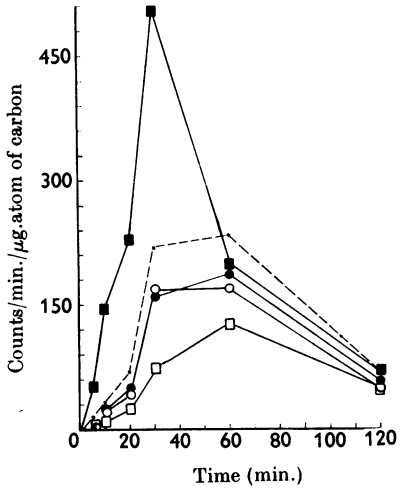

Fig. 1. Rate of incorporation of ^{14}C into amino acids of rat brain after subcutaneous injection of [U-^{14}C]glucose. ■——■, Alanine; ○——○, aspartate; ●——●, glutamate; □——□, glutamine; ●- - -●, γ-aminobutyrate.

decarboxylated. In Expt. 1 the specific radioactivity of γ-aminobutyrate carbon was initially greater than that of glutamate (Fig. 1). In another group of animals (Expt. 2) the specific radioactivities of γ-aminobutyrate were lower than those of glutamate in brain. The observed values for the two amino acids were respectively: 7·9, 10·4 at 5 min.; 22·5, 28·2 at 10 min.; 81·8, 96·9 at 20 min.; 163·1, 203·9 at 30 min.; 125·8, 142·1 at 60 min.; 87·8, 105·1 at 120 min. The specific radioactivities

of these two amino acids reached a peak between 30 and 60 min. after the injection.

The specific radioactivity of glutamine was approximately 50% of that of glutamate during the first 30 min. but later the two amino acids became nearly equally labelled. The specific radioactivity of aspartate was similar to that of glutamate at all times.

Liver. On a fresh-weight basis the total radioactivity in the perchloric acid extract was at first greater in liver than in brain (cf. Tables 1 and 2); at 20 min. the radioactivity of the two tissues was nearly equal and later it was lower in liver than in brain. As found previously (Vrba *et al.* 1962; Gaitonde *et al.* 1964) only a fraction (17%) of the total ^{14}C in the perchloric acid extract of liver was accounted for by ^{14}C-labelled amino acids; most of the residual radioactivity was present in the glucose fraction. Concomitant with the marked decrease in the total radioactivity of the liver there was also a decrease in the radioactivity of the amino acids, but the proportion of ^{14}C remaining in the amino acids had increased from 17% to 37% (Table 2). Previous studies showed that during this period (30–60 min.) there was no increase in the specific radioactivity of liver proteins (Vrba *et al.* 1962); therefore the observed changes might be attributed to rapid redistribution of labelled glucose from liver to other tissues of the rat.

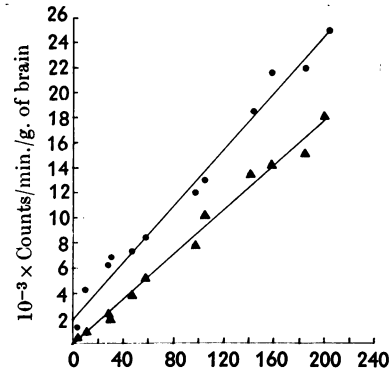

Fig. 2. Specific radioactivity of glutamate in brain as a function of total radioactivity in acid-soluble or amino fraction of brain after subcutaneous injection of glucose. ●, Acid-soluble fraction; ▲, amino acid fraction.

The radioactivity in alanine increased with time after injection; it reached its highest value at 60 min. and then decreased (Table 2). Other amino acids showed an increase in their ^{14}C content at all times after the injection. At 30 min. the distribution of ^{14}C in amino acids as percentages of the total radioactivity in acid-soluble metabolites of liver was: alanine 3%, glutamate 5·2%, glutamine 5·3% and aspartate 2·6%; serine and glycine each contained approximately 1% of the radioactivity at 60 and 120 min.

All amino acids examined reached the highest specific radioactivities at 30 min. after the injection of glucose. The results are shown in Fig. 3. As in the brain, the specific radioactivity of alanine in the liver was greater than that of other amino acids at all times during the first 20 min. Alanine, like aspartate, was rapidly labelled; its specific radioactivity was higher than that of glutamate at all

Table 2. *Distribution of* ^{14}C *in amino acids of rat liver after subcutaneous injection of* [*U*-^{14}C]*glucose*

Percentages are with reference to total radioactivity in the extract. Serine and glycine each contained 0·1–0·6% radioactivity during the first 30 min. and 1–1·2% at 60 and 120 min. after the injection.

Time after injection (min.)	Expt. no.	Counts/min./g. of fresh tissue: Perchloric acid extract	Counts/min./g. of fresh tissue: Amino acids	^{14}C in amino acids (% of total radioactivity)	Percentage distribution of ^{14}C: Alanine	Glutamate	Glutamine	Aspartate	Total recovered
5	1	5310	230	4·4					
	2	15850	850	5·4	2·0	0·9	0·5	0·5	4·1
10	1	12410	850	6·9					
	2	12630	820	6·5	1·9	1·4	0·7	0·9	5·1
20	1	10560	1270	12·0					
	2	16520	2360	14·3	1·9	4·8	3·3	1·7	12·4
30	1	18900	3320	17·6					
	2	18910	3550	18·8	3·0	5·2	5·3	2·6	17·0
60	1	9700	2460	25·4					
	2	9170	2440	26·6	4·1	7·1	9·0	2·2	24·4
120	1	3950	1380	34·9					
	2	4940	1810	36·7	1·7	8·0	12·5	3·9	28·3

times. The specific radioactivity of glutamine was, as in brain, lower than that of glutamate. The labelling pattern of individual amino acids of liver was confirmed in a separate group of animals (Expt. 2).

Free and bound amino acids of rat brain

The uncombined amino acids in the rat brain were determined under two conditions: (*a*) rapid removal of the brain after decapitation and immediate freezing of the brain in liquid nitrogen; (*b*) removal of the brain and immediate homogenization in saline or sucrose medium at room temperature followed by separation into supernatant and residue fractions. The latter procedure required approximately 10 min. from the time of decapitation of the animal until the suspension was cooled in the centrifuge and a further 20 min. before protein precipitation with perchloric acid.

The amino acid amounts found are given in Table 3. Except for glutamine and γ-aminobutyrate the values obtained under condition (*a*) are considerably lower than those under condition (*b*). The general increase in amino acids observed under condition (*b*) suggests that there was some proteolysis. Proteinases active at neutral pH have been shown to be present in rat brain (Ansell & Richter, 1953; Lajtha, 1961). The γ-aminobutyrate concentrations have been found to be affected by the conditions under which the tissue is isolated and treated before deproteinization (Lovell & Elliott, 1963). The γ-aminobutyrate values obtained in the present work under condition (*a*) were lower than those previously reported, but strain differences are known to occur (e.g. Lovell & Elliott, 1963). They are also lower than those reported by Cremer (1964) from rats of the same strain as used in this work; this is probably due to differences in the conditions used before deproteinization of the tissue. The increase in γ-aminobutyrate under condition (*b*) shown in Table 3 confirms earlier observations of a rapid accumulation of γ-aminobutyrate in brain homogenates (R. A. T. Khan & K. A. C. Elliott, unpublished work).

The results given in Table 3 are in agreement with earlier findings obtained by different methods and with different strains of rats, that part of the γ-aminobutyrate and other amino acids in brain homogenates is 'free' (supernatant solution) and a part is 'bound' in a particulate fraction (Elliott & van Gelder, 1958; R. A. T. Khan & K. A. C. Elliott, unpublished work). The proportions found in the 'bound' form in saline medium were about the same for alanine, glutamate and glutamine. The proportion of 'bound' aspartate was consistently higher and that of γ-aminobutyrate higher still. After suspension of brain tissue in iso-osmotic sucrose solution the amounts in the 'bound' form were in all cases lowered, especially that of γ-aminobutyrate and glutamine.

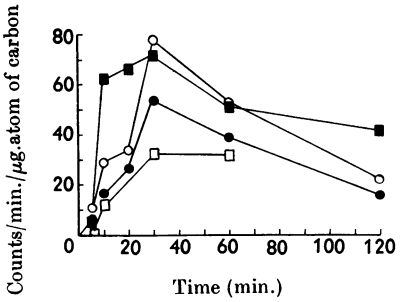

Fig. 3. Rate of incorporation of ^{14}C into amino acids of rat liver after subcutaneous injection of [U-^{14}C]glucose. ■, Alanine; ○, aspartate; ●, glutamate; □, glutamine.

Table 3. *Amino acid content of perchloric acid extract and of supernatant ('free') and residue ('bound') fractions of a saline–phosphate extract of rat brain*

(*a*) The frozen brain was extracted with perchloric acid; (*b*) the saline–phosphate extract of brain was centrifuged to obtain supernatant and residue fractions, which were then deproteinized with perchloric acid.

		(*b*)			
			Bound		
	(*a*) Total	Total (free + bound)	Saline suspension		Sucrose suspension
Amino acid	(μmoles/g.)	(μmoles/g.)	(μmoles/g.)	(%)	(%)
Alanine	0·6	1·1	0·3	27	23
Aspartate	2·5	4·5	2·0	44	33
Glutamate	9·7	12·2	3·7	30	23
Glutamine	4·4	4·3	1·3	30	20
γ-Aminobutyrate	1·3	1·7	0·9	53	32

Table 4. *Relative specific radioactivities of 'free' and 'bound' amino acids after intravenous injection of [U-^{14}C]glucose*

Mean specific radioactivity of alanine was taken as 1.

Time after injection (min.) ...		2·5			5·0			30		120	
Radioactivity injected (μc)		20	20	20	10	10	20*	10	10	20	20
Total radioactivity in brain ($10^{-2}\times$ counts/min./g.)		277	206	348	196	207	500	357	450	152	163
Total radioactivity in amino acids analysed (% of total in brain)		36	24	34	46	42	42	26	28	30	42
Mean sp. radioactivity of alanine (counts/min./μg.atom of carbon)		362	210	211	192	114	537	52	125	33	91
Alanine	free	1·00	0·91	0·85	1·15	1·16	0·96	1·0	0·9	1·32	0·98
	bound	0·96	1·19	1·37	0·88	0·64	1·17	1·1	1·2	0·12	1·03
Aspartate	free	0·28	0·20	0·61	0·36	0·74	0·56	2·13	1·10	1·26	0·64
	bound	0·34	0·38	0·72	0·45	0·74	0·50	1·65	1·26	1·59	0·58
Glutamate	free	0·24	0·21	0·49	0·37	0·87	0·48	1·85	1·38	1·42	0·97
	bound	0·23	0·22	0·54	0·37	0·93	0·48	1·65	1·55	1·95	0·91
Glutamine	free	0·05	0·05	0·08	0·05	0·27	0·15	0·60	0·67	—	0·47
	bound	0·08	0·05	0·15	0·09	0·13	0·12	0·60	0·44	—	0·29
γ-Aminobutyrate	free	0·32	0·54	0·45	0·42	0·53	0·46	1·46	1·33	1·56	0·82
	bound	0·28	0·31	0·50	0·77	0·54	0·46	1·38	1·14	1·56	0·48

* Results in this column were obtained from brain suspension in 0·3 M-sucrose solution. All others were obtained from tissue suspended in saline–phosphate.

Incorporation of ^{14}C after intravenous injection of [U-^{14}C]glucose

The studies of the 'free' and 'bound' amino acids in rat brain raised the question whether these two pools of amino acids could be shown to differ metabolically *in vivo*. To test this possibility the specific radioactivity of individual amino acids was determined in the 'free' and 'bound' amino acid fractions obtained from the brains of rats after intravenous injection of [U-^{14}C]glucose. The amount of alanine in these pools was small (Table 3); initially it was the most highly labelled amino acid and probably it had a specific radioactivity similar to that of the glucose.

The radioactivity per carbon atom of the other amino acids is given relative to that in alanine (Table 4). There appears to be no consistent difference, greater than the probable errors involved, between the relative specific radioactivities of 'free' and 'bound' amino acids. This is true even when the rather more firmly occluded fraction of the 'bound' amino acid which remains after suspension in sucrose solution is compared with the others.

The labelling pattern of the amino acids after intravenous injection was very similar to that obtained after subcutaneous injection (cf. Fig. 1 and Table 4), although the rapidity of labelling was much higher. The specific radioactivity of γ-aminobutyrate and of aspartate in these experiments was initially somewhat higher than that of glutamate.

DISCUSSION

In previous studies it was shown that uniformly labelled glucose injected into rats and cats was rapidly incorporated into amino acids in the brain (Vrba *et al.* 1962; Gaitonde *et al.* 1964). In the present work the rate of entry of radioactive carbon into individual amino acids was determined. During the first 30 min. after subcutaneous injection there was a rapid increase in the radioactivity of alanine, glutamate, glutamine and γ-aminobutyrate in the brain (Table 1). The distribution pattern of ^{14}C at 30 min. expressed as a percentage of the total radioactivity of the acid-soluble metabolites of the brain was: alanine 2%, glutamate 38%, glutamine 9%, aspartate 9% and γ-aminobutyrate 4%. Serine and glycine together contained less than 1% of the total radioactivity. Glutamate was thus the main labelled product accumulating in the brain after injection of [U-^{14}C]glucose. Indeed the total radioactivity of the acid-soluble metabolites or of the amino acid fraction was proportional to the specific radioactivity of the glutamate (or aspartate) in the brain (Fig. 2).

The results of the experiments in which labelled glucose was administered intravenously illustrate the extreme rapidity with which glucose carbon enters the amino acids. Within 2·5 min. about 24–36% of the total radioactivity in the brain was present in the five amino acids studied. In the present investigation labelled amino acids were isolated and their specific radioactivity was deter-

mined. The specific radioactivity of γ-aminobutyrate was higher than or similar to that of glutamate soon after the subcutaneous or intravenous injection of glucose. Since γ-aminobutyrate is formed by decarboxylation of glutamate, the specific radioactivity of γ-aminobutyrate might have been expected to be lower than that of glutamate soon after the injection. The observed finding of a rapid labelling of γ-aminobutyrate carbon suggests that γ-aminobutyrate is formed from a pool of highly labelled glutamate (active glutamate pool) derived from highly labelled α-oxoglutarate (active α-oxoglutarate pool). The active glutamate pool which is formed from the injected [U-^{14}C]glucose must be in a compartment different from that containing the main glutamate pool of the brain. The small active pool, which may be located in the mitochondria, contains aspartate, γ-aminobutyrate and some glutamine as well as glutamate. During the extraction of the tissue the active glutamate will be diluted by the main tissue pool. Further observations which support this view were made in the course of the present experiments (M. K. Gaitonde, unpublished work). Some estimates of the specific radioactivities of amino acids of rat brain after administration of [U-^{14}C]glucose *in vitro* and *in vivo* have been reported by Cremer (1964), who also suggested that labelling of γ-aminobutyrate observed in her studies might have occurred from an active glutamate pool. Evidence for the 'compartmentation' of glutamate *in vivo* has also been obtained by other workers in experiments involving injection of [^{14}C]glutamate (Berl, Lajtha & Waelsch, 1961) or of [2-^{14}C]pyruvate (Albers, Koval, McKhann & Ricks, 1961). It can be calculated from the data in Table 1 that the radioactivity in γ-aminobutyrate accounted for 11–18% of the active glutamate pool.

Direct carbon dioxide fixation has been estimated to contribute only about 10% of the labelling of aspartate in brain (Moldave, Winzler & Pearson, 1953; Berl, Takagaki, Clarke & Waelsch, 1962; McMillan & Mortensen, 1963; Otsuki, Geiger & Gombos, 1963). The main source of radioactivity of the aspartate isolated in the present studies would then be by oxidation of [^{14}C]succinate derived from α-oxo[^{14}C]glutarate or via oxidation of γ-amino[^{14}C]butyrate. The pool size of the intermediates of the citric acid cycle from succinate to oxaloacetate is about 2·4 μmoles/g. wet wt. (Frohman, Orten & Smith, 1951) and those of aspartate and glutamate about 2·5 and 9·7 μmoles/g. wet wt. respectively. The effective dilution of labelled carbon of aspartate would be approximately 50% of that of the glutamate carbon so that the specific radioactivity of aspartate might be expected to be considerably higher than that of glutamate. In contrast the observed specific radioactivity of aspartate was about equal to or less than that of glutamate: this indicates that considerable dilution of aspartate carbon had occurred *in vivo*. The source of this dilution is most likely the large tissue pool of glutamate of low specific radioactivity.

The degree of labelling in glutamine was lower than in glutamate in the brain. This could be due to slow formation of new glutamine from the active glutamate pool or formation from the less active glutamate pool. That a part of the labelled glutamine in these experiments was formed directly from the active glutamate pool is suggested also by the observations of M. K. Gaitonde (unpublished work).

Experiments in which the specific radioactivities of individual amino acids in the 'free' and 'bound' states were determined after rapid labelling by intravenous injection of [U-^{14}C]glucose showed no significant difference in their specific radioactivities (Table 4).

The relatively low incorporation of ^{14}C in the amino acid fraction of liver confirms the results of previous studies on the rat and cat (Vrba *et al.* 1962; Gaitonde *et al.* 1964). The distribution pattern of ^{14}C in amino acids was: alanine 3%, glutamate 5·2%, glutamine 5·3% and aspartate 2·6%. This is in sharp contrast with the brain, where the specific radioactivities of the individual amino acids were considerably higher. The observed difference in the metabolism of [U-^{14}C]glucose in the two organs is probably due to differences in glucose pools, amino acid pools and rates of glycolysis (Gaitonde *et al.* 1964).

In liver the specific radioactivity of glutamate was higher than that of glutamine but lower than that of aspartate. These findings may also be explained on the assumption that glutamine in the liver is also formed by a slow process from the active glutamate pool or from a less active glutamate pool. The higher specific radioactivity of aspartate in the liver is probably an indication that a considerable proportion of the ^{14}C enters aspartate by direct fixation of carbon dioxide with pyruvate (Berl *et al.* 1962).

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