Rate of Utilization of Glucose and 'Compartmentation' of α-Oxoglutarate and Glutamate in Rat Brain

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1. The rate of incorporation of ^{14}C into pyruvate, α-oxoglutarate, lactate and glucose of rat tissues was measured after the subcutaneous injection of uniformly labelled glucose. 2. In rat brain the specific radioactivities of lactate and glucose were similar to that of alanine. In liver the specific radioactivity of glucose was considerably higher than that of lactate or alanine. 3. The specific radioactivities of α-oxo acids of rat brain were lower than those of corresponding amino acids, alanine and glutamate. These findings have been explained in relation to metabolic compartments *in vivo*. 4. The approximate estimated rate of glucose utilization in rat brain *in vivo* is 0·96 μmole/g. of brain/min.

A rapid rate of entry of ^{14}C into the amino acids of the brain was previously observed after the administration of uniformly labelled glucose *in vivo* (Gaitonde, Marchi & Richter, 1964). The specific radioactivity of γ-aminobutyrate was similar to or higher than that of glutamate, and it was suggested that labelled γ-aminobutyrate is probably formed in an active glutamate pool situated in the mitochondria (Gaitonde, Dahl & Elliott, 1965). In the present investigation further information on the metabolic pools *in vivo* has been obtained by measuring the radioactivity of the α-carboxyl carbon (C-1) of individual amino acids and the specific radioactivities of other metabolites, including α-oxoglutarate, pyruvate and lactate. Also, by measuring the specific radioactivity of glucose in the brain during the first 5 min. after the injection of uniformly labelled glucose, a tentative estimate has been made of the rate of utilization of glucose in the brain *in vivo*. A part of the data relating to individual amino acids was reported by Gaitonde *et al.* (1965).

EXPERIMENTAL

Animals. Male rats derived from a Wistar albino strain weighing 100–110 g. were injected subcutaneously with 10 μc of [U-^{14}C]glucose (The Radiochemical Centre, Amersham, Bucks.) contained in 1·02 mg. of glucose in 0·5 ml. solution. The animals were decapitated at different times after the injection. The brain, which was removed immediately, and liver were blotted on filter paper and dropped into liquid nitrogen. The frozen tissues were dispersed in ice-cold 6% (w/v) perchloric acid. Blood samples of decapitated animals were collected in beakers containing 0·2 ml. of heparin solution, mixed and pipetted into perchloric acid. The perchloric acid extracts were prepared and neutralized as described by Gaitonde *et al.* (1965).

Fractionation of perchloric acid extract. An account of the methods is given by Gaitonde *et al.* (1964). From the neutralized perchloric acid extract the following main fractions were obtained: (*a*) amino acid fraction, (*b*) glucose fraction and (*c*) carboxylic acid fraction. The amino acids and other cations were absorbed on a column of Zeo-Karb 225 (H^+ form) and subsequently eluted with 1N-ammonia solution. The solution of compounds that were not absorbed on the Zeo-Karb column was collected together with a water washing (30 ml.) of the resin. It was then passed directly through a column (15 cm.) of Dowex 1 (CO_3^{2-} form): glucose, inositol and other reducing compounds were collected in the effluent; the resin bed was then washed with water (30 ml.) and the washings were combined with the main effluent. The compounds absorbed by the resin were eluted with 0·1N-HCl (150 ml.): the carboxylic acid fraction was collected at the point when all the carbonate was exchanged with chloride and free HCl emerged from the column. The carboxylic acid fraction was divided into two equal parts: one was used for the isolation of lactate by paper chromatography and the other for the isolation of pyruvate and α-oxoglutarate as 2,4-dinitrophenylhydrazones.

Isolation of amino acids. The individual amino acids were isolated from the amino acid fraction by paper chromatography. After measurement of their specific radioactivities, which were reported by Gaitonde *et al.* (1965), the samples in the planchets were decarboxylated as described below.

Isolation of glucose. The glucose fraction containing free glucose, inositol and other reducing compounds was evaporated to dryness and dissolved in water. Under the conditions used, the recovery of known amounts of glucose (100 μg.) isolated after ion-exchange chromatography on Zeo-Karb 225 and Dowex 1 was 91–96%. The fraction obtained from liver contained glycogen, which was removed

by precipitation with 80% (v/v) ethanol. The reducing compounds were separated by one-dimensional paper chromatography (Whatman no. 4 paper) of duplicate samples with 90% (v/v) acetone in water. After locating the reducing compounds on one strip by the method of Trevelyan, Procter & Harrison (1950), the glucose spot from the other unstained strip was cut out, eluted in 6 ml. of water and portions were taken for the determination of ^{14}C (1·5 ml.) and glucose (1 ml.).

Glucose was determined by the following method, which is based on the procedures described by Guidotti, Colombo & Foà (1961) and Saifer & Gerstenfeld (1958). The enzyme reagent was freshly prepared by mixing a solution containing 10 mg. of glucose oxidase (type II, 16 units/mg.; Sigma Chemical Co., St Louis, Mo., U.S.A.) and 5 mg. of peroxidase (C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany) in 99 ml. of 0·06 M-NaH_2PO_4, pH 7·0, with 1 ml. of 1% (w/v) *o*-dianisidine in 95% (v/v) ethanol and filtered. *o*-Dianisidine was recrystallized repeatedly according to a method described for orcinol by Schneider (1957) until the final product was light yellow. The reagent (2·5 ml.) was added to 1 ml. of glucose sample. After incubation at 36° for 1 hr., 0·5 ml. of 0·8 N-H_2SO_4 was pipetted into the reaction mixture and the extinction was measured at 400 mμ. The mean extinction/0·1 μmole of glucose in a final volume of 4 ml., with cells of 1 cm. light-path, was 0·252. For quantitative determination of glucose known amounts of glucose were chromatographed under the same conditions used for tissue samples. The extinctions were corrected for paper blanks. Recovery of glucose after chromatography was 88–102% from Whatman no. 4 paper and 50–75% from Whatman no. 1 paper. In some later experiments Whatman no. 540 paper was used since (*a*) it gave excellent separation of several reducing compounds as discrete spots, (*b*) the recovery of added glucose was 95–100% and (*c*) the rate of movement of solvent front was much lower and the development of the chromatogram could conveniently be done overnight.

Isolation of lactate. The carboxylic acid fraction obtained from Dowex 1 (CO_3^{2-} form) resin was evaporated to dryness under reduced pressure at 50–60°. The residue in the flask was dissolved in 125 μl. of water. A portion (100 μl.) was transferred on to Whatman no. 1 paper (18 in. × 22 in.) for two-dimensional paper chromatography. The remaining solution (25 μl.) was spotted on an adjacent place as a marker for lactate. After development in the first direction with butan-2-ol–formic acid (85%)–water (5:1:1, by vol.) the chromatograms were dried, the marker strip was cut out and dipped in a solution of bromophenol blue and dimethyl yellow (Aronoff, 1956) to localize the spot of lactate, which, in the above solvent system, overlapped with succinate. The position of succinate–lactate on the unstained chromatogram was marked with a pencil, and a suitable width of the chromatogram was cut out. A mixture of succinate and lactate (100 μg. of each), which served as markers, was then spotted on the line adjacent but far below the localized position of the succinate–lactate spot. The chromatographic strip was developed in the second direction with propan-1-ol–aq. ammonia (sp.gr. 0·88)–water (16:1:3, by vol.). After thorough drying to remove ammonia, the marker strip of succinate and lactate was cut out and dipped in the indicator solution to localize the position of lactate, which moved much faster than succinate.

From the unstained chromatogram the lactate spot was cut out and eluted with 6 ml. of water. A portion (1·5 ml.) of the eluate was transferred into a planchet for the assay of ^{14}C and the remaining solution (3–3·5 ml.) was used for determination of lactate by the method of Barker & Summerson (1941). Paper blanks were included for each set of determinations.

Isolation of pyruvate and α-oxoglutarate. From the acid extract hydrazones were prepared by adding 2 ml. of 0·2% 2,4-dinitrophenylhydrazine in 2 N-HCl and extracted according to the procedure of El Hawary & Thompson (1953). The hydrazones were extracted with twice the volumes of ethyl acetate and sodium carbonate recommended by these authors. Paper chromatography of the hydrazones with butan-1-ol–ethanol–0·2 N-NH_3 (7:2:1, by vol.) showed four spots, one corresponding to α-oxoglutarate, two to pyruvate and the fourth between α-oxoglutarate and the upper spot of pyruvate. The identity of the fourth spot is not known; it was weakly radioactive.

The spots of α-oxoglutarate and the combined spots of pyruvate were eluted with 5 ml. of ethanol and 0·5 ml. of 0·5 N-NH_3. After collecting the solution, the paper strips were repeatedly washed with 50% (v/v) ethanol to complete the elution. The combined eluates were evaporated to dryness under reduced pressure (below 40°) and transferred into planchets. After measurement of radioactivity the

Table 1. *Amounts of glucose and α-oxo acids in rat blood and brain*

Tissue	Concn. ± S.D. (μg./g. fresh wt. or ml.) Glucose	Pyruvate	α-Oxoglutarate	Reference
Blood	590–1100*			Gey (1956)
		8·4 ± 1·4	6·5 ± 2·2	Goodwin & Williams (1952)
		21·7	7·2	Cavallini & Frontali (1954)
		22·0	4·5	LePage (1950)
	830 ± 83	4·9 ± 1·5	2·3 ± 0·5	Present work
Brain	80			Gey (1956)
		9·6	2·9	Cavallini & Frontali (1954)
		7·7	12·5	LePage (1950)
	39 ± 6	5·6 ± 1·2	1·7 ± 0·1	Present work

* Plasma.

hydrazones were dissolved in 1ml. of 10% Na_2CO_3–2N-NaOH (2:1, v/v). Amounts of pyruvate and α-oxoglutarate in the tissue were determined from known amounts of hydrazones of pyruvate and α-oxoglutarate prepared and chromatographed under the same conditions. The estimated values for the tissues were not corrected for small losses during the isolation of hydrazones by solvent extraction.

Decarboxylation of amino acids. After measurement of ^{14}C the amino acids were decarboxylated by adding 0·5ml. of buffered (pH5·0) 0·5% ninhydrin solution (Gaitonde, 1961) to each planchet. The reaction was allowed to proceed at room temperature for 24hr. in the fume cupboard and the dry planchets were assayed for residual ^{14}C of the aldehyde remaining in the planchet. Under these conditions uniformly labelled [^{14}C]glutamate (and presumably [^{14}C]-glutamine) showed 18–20% loss of the radioactivity. Since the aldehyde formed from glutamate is non-volatile (Virtanen, Laine & Toivonen, 1940) the loss of ^{14}C must be attributed to decarboxylation of C-1 of glutamate. Uniformly labelled aspartate gave a residual radioactivity of 66%, which in agreement with the findings of Virtanen *et al.* (1940) suggested that 10% of the radioactivity of aspartate was lost as volatile aldehyde. These results are also in agreement with the findings of Moldave, Winzler & Pearson (1953), who, using similar conditions, concluded that only C-1 of aspartate was decarboxylated with ninhydrin. However, under the conditions employed by Van Slyke, Dillon, MacFadyen & Hamilton (1941), 1mol. of aspartate liberated 2mol. of CO_2 by reaction with ninhydrin.

Measurement of radioactivity. All samples were collected in planchets and counted at infinite thinness in a Nuclear–Chicago gas-flow counter by using a Micromil window, as described by Vrba, Gaitonde & Richter (1962). The results were corrected for background counting rate.

RESULTS

The glucose contents of brain and blood given in Table 1 were obtained from rats after the subcutaneous injection of 1mg. of glucose containing 10μc of [U-^{14}C]glucose. Gey (1956) has reported values of 80μg./g. of brain and 590–1100μg./ml. of blood plasma of starved male albino rats.

The total ^{14}C content (counts/min./ml.) and the specific radioactivity of glucose (counts/min./μg.atom of carbon) in blood reached a maximum value at 30min. (Fig. 1). In previous studies (Gaitonde *et al.* 1965) it was shown that the total radioactivity in the perchloric acid extract and in the amino acid fraction of brain and liver was also maximal at 30min.: the results given in Fig. 2 showed that the specific radioactivity of glucose in these organs was also maximal at this time. The specific radioactivity of glucose carbon was lower in brain than in blood during the first 30min. and later it was similar in both tissues. It was considerably lower in liver than in blood except at 120min.: similar values have been obtained by C. J. Threlfall (unpublished work). The results of experiments of short duration showed that the

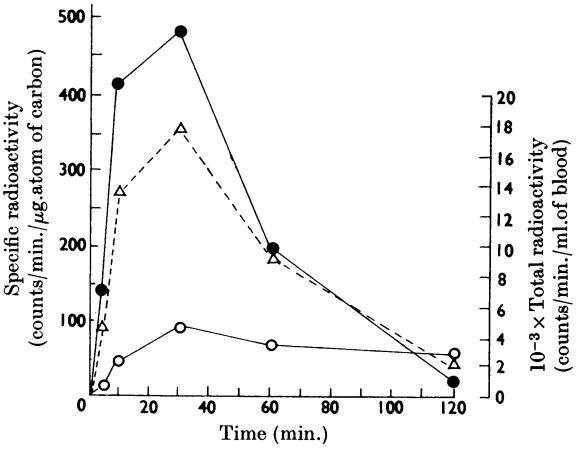


Fig. 1. Rate of entry of ^{14}C into blood and specific radioactivities of blood glucose and lactate after the subcutaneous injection of [U-^{14}C]glucose. ●, Glucose; ○, lactate; △, total ^{14}C (counts/min./ml.) of blood.

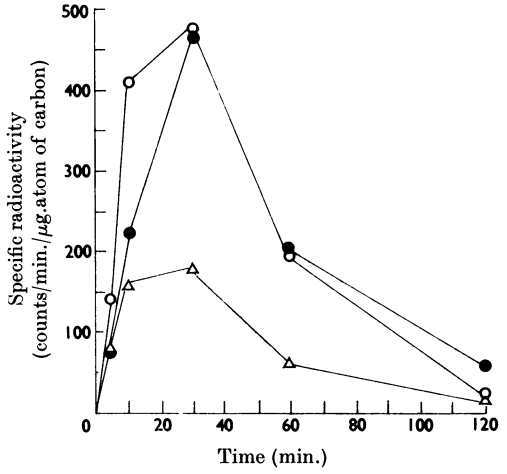


Fig. 2. Specific radioactivities of glucose in rat tissue after the subcutaneous injection of [U-^{14}C]glucose. ●, Brain; ○, blood; △, liver.

greater fraction of the radioactivity in rat brain was present in metabolites other than amino acids and glucose (Table 2).

In brain the specific radioactivities of lactate and alanine ran closely parallel to that of glucose. At 30min. the specific radioactivities of the three compounds were very similar and at 60min. they reached the same value (Fig. 3). In another series of experiments the specific radioactivities of alanine carbon were considerably lower than those of

Table 2. *Uptake of* ^{14}C *in rat brain during the first 5 min. after the injection of* [*U*-^{14}C]*glucose*

Experimental details are given in the text.

Time after injection (min.)	Radioactivity (counts/min./g. of brain)		Percentage distribution	
	Perchloric acid extract	Amino acids	Amino acids	Glucose
2	248	37	15	7
2	284	55	19	6
4	6008	1026	17	2
4	5336	1307	24	3
5	1430	276	19	8
5	4200	966	23	8

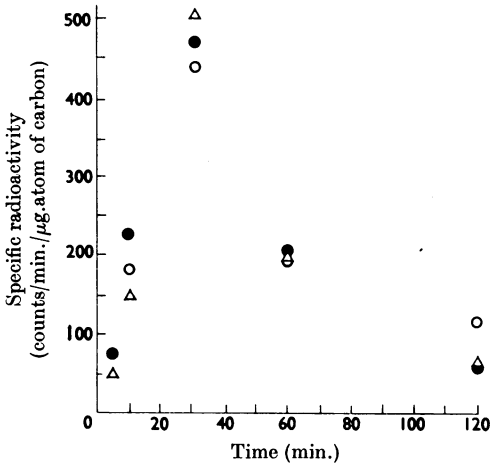


Fig. 3. Rate of entry of ^{14}C into metabolites of rat brain after the subcutaneous injection of [U-^{14}C]glucose. ●, Glucose; ○, lactate; △, alanine.

glucose carbon in brain. The observed values (counts/min./μg.atom of carbon) for alanine and glucose respectively were as follows: 56 and 164 at 5 min.; 86 and 175 at 10 min.; 209 and 419 at 30 min.; 141 and 190 at 60 min.; 68 and 102 at 120 min. after the injection of [U-^{14}C]glucose. The specific radioactivities of lactate and alanine in liver were also similar but they were considerably lower than that of glucose (Fig. 4). Lactate in blood (Fig. 1) showed a specific radioactivity intermediate between the values obtained for brain and liver.

Specific radioactivities of pyruvate and α-oxoglutarate. The concentrations of α-oxo acids determined after their isolation as 2,4-dinitrophenylhydrazones are given in Table 1. The estimated values are based on the extinctions of

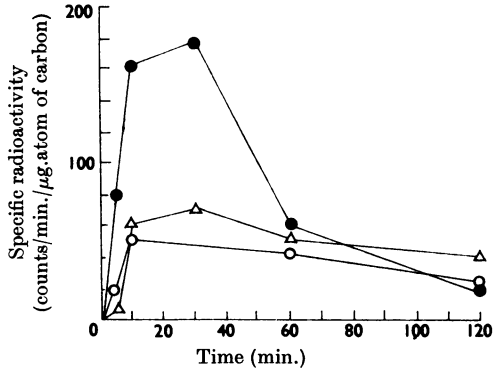


Fig. 4. Rate of entry of ^{14}C into metabolites of rat liver after the subcutaneous injection of [U-^{14}C]glucose. ●, Glucose; ○, lactate; △, alanine.

known amounts of the hydrazones eluted from paper chromatograms. They are lower than those reported by other workers (Table 1).

The specific radioactivity of pyruvate was considerably lower than that of lactate or alanine in the rat brain (Table 3). At 5 min. after injection, the degree of labelling in α-oxoglutarate was lower than that of pyruvate, but at times after 10 min. it showed a value that was similar to or higher than that of pyruvate. The specific radioactivity of α-oxoglutarate was of the same magnitude as that of glutamate during the first 10 min., but later the specific radioactivity of glutamate far exceeded that of α-oxoglutarate in the brain: the two compounds reached an isotopic equilibrium at 120 min. (Table 3). The degree of labelling of the two α-oxo acids in blood was also low (Table 3).

Radioactivity of carboxyl carbon of amino acids. The entry of ^{14}C of the injected [U-^{14}C]glucose through the citric acid cycle would be expected to result in the non-uniform labelling of amino acids except alanine. If all the carbon atoms of the whole of the amino acids in the tissue were labelled uniformly after injection of [U-^{14}C]glucose, the radioactivity in the α-carboxyl carbon atom would be expected to be 20% of the total radioactivity in glutamate and glutamine and 25% in aspartate. The results given in Fig. 5 showed that the labelling of the α-carbon atoms in these amino acids was not uniform. The radioactivity of C-1 of glutamate was approx. 20% of the total radioactivity in the amino acid from 30 to 120 min.; the C-1 of glutamine generally contained more radioactivity than that of glutamate in brain. Aspartate lost 40–50% of the radioactivity on treatment with ninhydrin. There is, however, some uncertainty about which carboxyl carbon atom(s) of aspartate are lost by decarboxylation (see the Experimental section).

Table 3. *Specific radioactivities of α-oxo acids and amino acids of rat tissues*

Experimental details are given in the text. The results on the labelling of amino acids were given by Gaitonde *et al.* (1965).

Time after injection (min.)	Specific radioactivity (counts/min./μg.atom of carbon)					
	Brain				Blood	
	Pyruvate	Alanine	α-Oxoglutarate	Glutamate	Pyruvate	α-Oxoglutarate
5	19	51	2	4	9	3
10	23	144	18	25	11	—
20	15	228	28	47	21	—
30	41	503	74	158	48	33
60	21	197	42	185	—	—
120	0	68	56	57	11	11

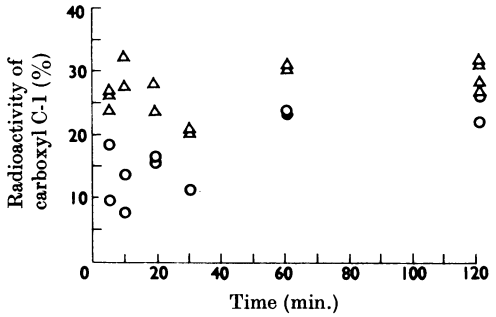


Fig. 5. Rate of labelling of carboxyl carbon of amino acids of rat brain after the injection of [U-^{14}C]glucose. ○, Glutamate; △, glutamine.

Table 4. *Rate of utilization of glucose in brain*

The values obtained for $-Q_{O_2}$ by previous workers was used to calculate the amount of glucose consumed assuming the dry wt. to be 21% of the fresh wt.

Species	Glucose utilization (μmole/g. of fresh brain/min.)		Reference
	in vitro[a]	*in vivo*[b]	
Man	0·12	0·28	[a]See Elliott & Wolfe (1962) [b]Kety (1957)
Monkey	0·15	0·29	See Elliott & Wolfe (1962)
Dog	0·39	0·34	See Krebs (1950)
Cat	0·40	0·31–0·39	See Elliott & Wolfe (1962)
Rat	0·50	0·96	[a]Krebs (1950) [b]Present work

DISCUSSION

Rate of utilization of glucose in vivo. A rapid uptake of ^{14}C in the brain and liver was shown previously in experiments in which uniformly labelled glucose was injected into animals. However, the metabolic fate of the ^{14}C differed in the two organs: whereas in brain more than 70% of the radioactivity was found in the amino acid fraction, in liver the amino acid fraction contained only 17% of the radioactivity at 30min. after injection (Vrba *et al.* 1962; Gaitonde *et al.* 1964; Gaitonde *et al.* 1965). These differences were discussed in relation to the operation of several factors including transaminase activities, glucose pools, amino acid pools and rates of glycolysis *in vivo* (Gaitonde *et al.* 1964; Balázs & Haslam, 1965).

The specific radioactivity of glucose in blood, liver and brain reached maximal values at 30min. after the subcutaneous injection of [U-^{14}C]glucose. In brain the specific radioactivities of glucose, lactate and alanine at 30min. were similar: this gave evidence of a rapid rate of utilization in the brain of the injected [U-^{14}C]glucose. In contrast, in liver the specific radioactivity of glucose was considerably higher than that of alanine or lactate. This could not be due to isotopic dilution of lactate in the liver by lactate entering from the blood, since the specific radioactivity of the blood lactate was similar to or higher than that in liver. Assuming that the injected glucose mixes rapidly with that in the endogenous glucose pool of the liver, the present findings suggest that the rate of pyruvate production was much lower in liver than in brain. The fact that the ^{14}C content of the amino acids was greater in brain than in liver can be attributed to the relative amounts of labelled pyruvate and α-oxoglutarate formed in the two tissues. Moreover, though the main substrate for oxidation in brain is glucose, the substrates for oxidation in liver include fatty acids and lactate entering from the blood.

Assuming that glucose is utilized in brain mainly via the Embden–Meyerhof pathway, in experiments of short duration (2–5min.) the amount of radioactive carbon dioxide lost would be approx. 33% of the pyruvate entering the citric acid cycle.

Since there is an increase in the ^{14}C content of amino acid, especially of glutamate and aspartate, during the first 30min. after the injection of [^{14}C]glucose, losses of ^{14}C as carbon dioxide would be expected to occur by the further oxidation of [^{14}C]glutamate via α-oxoglutarate, and in experiments of short duration (2–5min.) this loss may be considered as insignificant: the radioactivity lost by decarboxylation of pyruvate would thus be considered approx. 50% of that retained in amino acids. Also, the ^{14}C content of lipids, proteins and nucleic acids in the tissue residue after perchloric acid extraction was not greater than 1% of the total radioactivity in the brain during the first 30min. after the injection of ^{14}C glucose. Therefore the radioactivity of glucose metabolized may be equated to the radioactivity retained in the metabolites other than glucose in the perchloric acid extract, plus the radioactivity lost as carbon dioxide by the decarboxylation of pyruvate and the radioactivity of metabolites returned to the blood stream. In experiments of short duration the radioactivity of metabolites returned to the blood may be considered negligible. Hence:

$$m.S_t = \frac{C}{t} + \frac{0{\cdot}5A}{t} = \frac{R}{t} \text{ or } m = \frac{[R/t]}{S_t}$$

where m is the rate of utilization in μg.atoms of carbon of glucose/g. of brain/min., S_t is the observed specific radioactivity of glucose carbon (counts/min./μg.atom of carbon) in the brain at time t min., and C and A are the radioactivities in counts/min. of metabolites other than glucose and of total amino acids respectively in perchloric acid extract of 1g. of brain at time t. The mean slope of R/t plotted against S_t from Fig. 6 is 5·76 and hence m, the rate of utilization of glucose carbon, is 5·76 μg.-atoms of carbon or 5·76/6 = 0·96 μmole of glucose/g. of brain/min. (see Table 4).

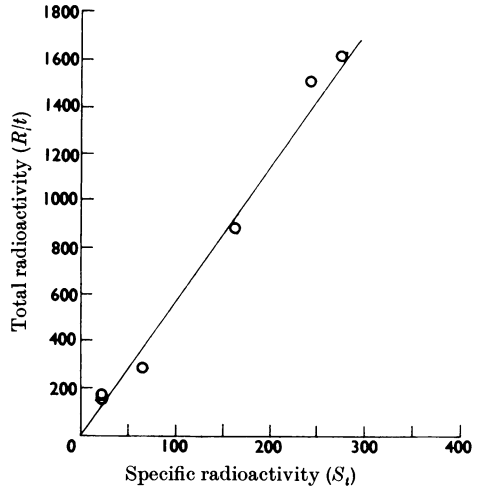
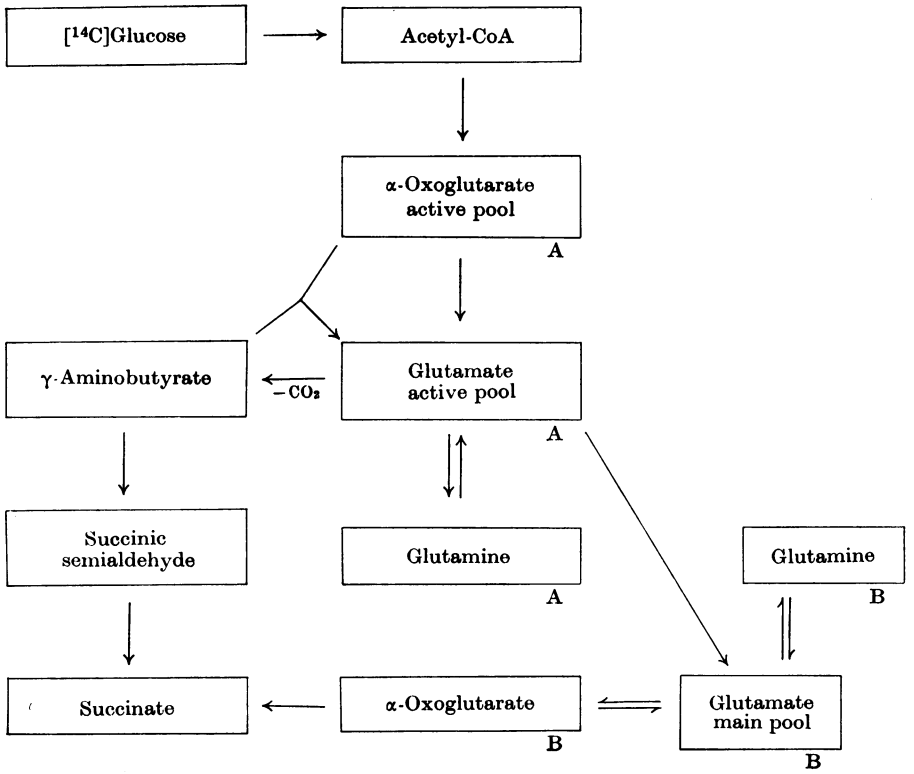


Fig. 6. Specific radioactivity of glucose carbon in brain as a function total radioactivity (R/t) per g. of brain during the 2–5 min. after the subcutaneous injection of [U-^{14}C]glucose. R = counts/min. of perchloric acid extract plus ½ × counts/min. of total amino acids of 1g. of brain at time t min. S_t = counts/min./μg.atom of glucose carbon at time t.

'Compartmentation' of α-oxoglutarate and glutamate. From the observed rapid rate of entry of ^{14}C into γ-aminobutyrate in the brain after the injection of uniformly labelled glucose, it was suggested that γ-aminobutyrate is formed from an active glutamate pool derived from an active α-oxoglutarate pool in the mitochondria (Gaitonde *et al.* 1965). The specific radioactivity of α-oxoglutarate therefore would be expected to be higher than that of glutamate or γ-aminobutyrate. In contrast, the results of the present investigations showed that the specific radioactivity of α-oxoglutarate was somewhat lower than that of glutamate during the first 10min. and considerably lower during subsequent times: at 120min. the two components had reached isotopic equilibrium. These findings indicate that there are at least two metabolic pools of α-oxoglutarate corresponding to the two metabolic pools of glutamate.

The observed labelling pattern of amino acids reported previously, and of α-oxoglutarate reported in this work, can be related to metabolic pools *in vivo* that may be represented as shown in Scheme 1. The two pools of α-oxoglutarate suggested are: pool A, active ^{14}C-labelled α-oxoglutarate from which active glutamate is derived; pool B, an α-oxoglutarate pool that is probably in direct equilibrium with the main glutamate pool of the brain. Labelled glucose from the blood first enters pool A. Labelled glutamate from the active pool A is (*a*) partly decarboxylated to γ-aminobutyrate, (*b*) partly amidated to glutamine and (*c*) partly mixed with the main glutamate pool B of the brain. The specific radioactivity of glutamine was considerably lower than that of glutamate (Gaitonde *et al.* 1965) but, as shown in the present work, the radioactivity of C-1 when compared with the residual radioactivity (C-2 to C-5) was greater in glutamine than in glutamate in brain. This might be attributed to the existence of two pools of glutamine synthesis: a mitochondrial pool A associated with active glutamate pool A and a cytoplasmic pool B probably associated with the endoplasmic reticulum. This view is in accord with the two sites of glutamine synthesis suggested from the results of experiments in which [^{14}C]glutamate (Berl, Lajtha & Waelsch, 1961) and $^{15}NH_4Cl$ (Berl, Takagaki, Clarke & Waelsch, 1962) were administered to animals.



Scheme 1. Representation of metabolic pool formed during glucose metabolism *in vivo*. Rapidly labelled pools after injection of [U-^{14}C]glucose are marked A; the main tissue pools are marked B. Different aspects of the 'compartmentation' of rapidly labelled glutamate, γ-aminobutyrate and glutamine have been discussed by Berl *et al.* (1961, 1962), Albers, Koval, McKhann & Ricks (1961), Cremer (1964), Gaitonde (1964) and Gaitonde *et al.* (1965).

The degree of labelling of aspartate was similar to that of glutamate in brain. A part of the ^{14}C incorporated into aspartate would be derived from succinate derived directly by oxidation of γ-aminobutyrate formed in active glutamate pool A and a part by oxidation of α-oxoglutarate. It was, however, indicated previously that a considerable dilution of aspartate carbon had occurred *in vivo*, and this was attributed to dilution of α-oxoglutarate pool (Gaitonde *et al.* 1965). The latter pool would correspond to α-oxoglutarate pool B shown in Scheme 1. The observed low specific radioactivity of the α-oxoglutarate pool might be expected if the main tissue glutamate pool B, which has been assumed to be in direct equilibrium with α-oxoglutarate pool B, is labelled by slow mixing of [^{14}C]glutamate from pool A.

In experiments *in vitro* with brain preparations and partly purified enzymes an isotopic exchange between ^{14}C-labelled α-oxoglutarate and non-labelled glutamate, or vice versa, has been reported to proceed to equilibrium within 10min. (Albers & Jakoby, 1960; Haslam & Krebs, 1963; Balázs & Haslam, 1965). It is not known to what extent such an isotopic exchange occurs *in vivo*, but it might very well operate to equilibrate α-oxoglutarate and glutamate in pool B (Scheme 1). The metabolic 'compartmentation' shown in Scheme 1 implies that exogenous glucose carbon is diluted by the main glutamate pool of brain before it is finally oxidized to carbon dioxide.

The degrees of labelling of lactate and alanine in brain were similar: this also gives evidence of the rapid metabolism of glucose in brain. Except at very early times after the injection of glucose, the specific radioactivity of pyruvate was less than 10% of the specific radioactivity of alanine. 'Compartmentation' of pyruvate in two separate pools such as that described for α-oxoglutarate cannot be excluded. It must be assumed that the rates of removal of pyruvate as lactate, alanine and acetyl-CoA and of α-oxoglutarate as glutamate are

so great that at any instant the accumulation of these highly labelled α-oxo acids is indeed negligible.

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REFERENCES

Albers, R. W. & Jakoby, W. B. (1960). In *Inhibition in the Nervous System and γ-Aminobutyric Acid*, p. 468. Ed. by Roberts, E. London: Pergamon Press Ltd.

Albers, R. W., Koval, G., McKhann, G. & Ricks, D. (1961). In *Regional Neurochemistry*, p. 340. Ed. by Kety, S. S. & Elkes, J. Oxford: Pergamon Press Ltd.

Aronoff, S. (1956). *Techniques of Radiobiochemistry*, p. 119. Ames: Iowa State College Press.

Balázs, R. & Haslam, R. J. (1965). *Biochem. J.* **94**, 131.

Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.

Berl, S., Lajtha, A. & Waelsch, H. (1961). *J. Neurochem.* **7**, 186.

Berl, S., Takagaki, G., Clarke, D. D. & Waelsch, H. (1962). *J. biol. Chem.* **237**, 2570.

Cavallini, D. & Frontali, N. (1954). *Biochim. biophys. Acta*, **13**, 439.

Cremer, J. E. (1964). *J. Neurochem.* **11**, 165.

El Hawary, H. F. S. & Thompson, R. H. S. (1953). *Biochem. J.* **53**, 340.

Elliott, K. A. C. & Wolfe, L. S. (1962). In *Neurochemistry*, 2nd ed., p. 177. Ed. by Elliott, K. A. C., Page, I. H. & Quastel, J. H. Springfield, Ill.: Charles C. Thomas.

Gaitonde, M. K. (1961). *J. Neurochem.* **8**, 234.

Gaitonde, M. K. (1964). *Biochem. J.* **90**, 42P.

Gaitonde, M. K., Dahl, D. R. & Elliott, K. A. C. (1965). *Biochem. J.* **94**, 345.

Gaitonde, M. K., Marchi, S. A. & Richter, D. (1964). *Proc. Roy. Soc.* B, **160**, 124.

Gey, K. F. (1956). *Biochem. J.* **64**, 145.

Goodwin, T. W. & Williams, G. R. (1952). *Biochem. J.* **51**, 708.

Guidotti, G., Colombo, J. P. & Foà, P. P. (1961). *Analyt. Chem.* **33**, 151.

Haslam, R. J. & Krebs, H. A. (1963). *Biochem. J.* **88**, 566.

Kety, S. S. (1957). In *Metabolism of the Nervous System*, p. 221. Ed. by Richter, D. London: Pergamon Press Ltd.

Krebs, H. A. (1950). *Biochim. biophys. Acta*, **4**, 249.

LePage, G. A. (1950). *Cancer Res.* **10**, 393.

Moldave, K., Winzler, R. J. & Pearson, H. E. (1953). *J. biol. Chem.* **200**, 357.

Saifer, A. & Gerstenfeld, S. (1958). *J. Lab. clin. Med.* **51**, 448.

Schneider, W. C. (1957). In *Methods in Enzymology*, vol. 3, p. 680. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.

Van Slyke, D. D., Dillon, R. T., Macfadyen, D. A. & Hamilton, P. (1941). *J. biol. Chem.* **141**, 627.

Virtanen, A. I., Laine, T. & Toivonen, T. (1940). *Hoppe-Seyl. Z.* **266**, 193.

Vrba, R., Gaitonde, M. K. & Richter, D. (1962). *J. Neurochem.* **9**, 465.