Conversion of Glutamate into Aspartate in Guinea-Pig Cerebral-Cortex Slices

By GERALD SIMON, M. M. COHEN AND J. F. BERRY

Department of Neurology, Presbyterian-St Luke's Hospital, University of Illinois, Chicago, Ill. 60612,
and Department of Neurology, University of Minnesota, Minneapolis, Minn., U.S.A.

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1. When guinea-pig cerebral-cortex slices were incubated with [U - ^{14}C]glutamate as substrate, the specific radioactivities of the citric acid-cycle intermediates were lower than that of the aspartate isolated from the same vessels. 2. Aspartate was significantly labelled when [5 - ^{14}C]glutamate was used as substrate and the aspartate contained almost no label when [1 - ^{14}C]glutamate was present as substrate. 3. When specifically labelled glutamate was used as substrate, the label was found in the isolated aspartate in the position that would be predicted by citric acid-cycle mechanisms. 4. The results are consistent with the theory of 'compartmentation' of amino acid metabolism.

Cohen, Simon, Berry & Chain (1962) reported that, when radioactive glutamate was incubated with brain slices for 1.5 hr., the aspartate that was isolated had a specific radioactivity at least four times that of the citric acid-cycle intermediates. As a result of this and other findings, it was postulated that some glutamate was converted into aspartate by a mechanism not involving the citric acid cycle. Haslam & Krebs (1963) maintained that the glutamate was converted into aspartate by citric acid-cycle mechanisms. The present study was undertaken to resolve these differences.

MATERIALS

Substrates. L-Glutamic acid was purchased from California Corp. for Biochemical Research (Los Angeles, Calif., U.S.A.). L-[U - ^{14}C]Glutamic acid and DL-[1 - ^{14}C]glutamic acid were purchased from Nuclear-Chicago Corp. (Des Plaines, Ill., U.S.A.). DL-[5 - ^{14}C]Glutamic acid was purchased from Niche Inc. (Bethesda, Md., U.S.A.). All substrates and radioactive tracers were purified as reported by Simon, Drori & Cohen (1967).

Reagents. Inorganic salts and organic solvents were Fisher certified reagent grade (Fisher Scientific Co., Pittsburgh, Pa., U.S.A.) or equivalent.

Before use, chloroform was distilled over K_2CO_3 , diethyl ether was washed with acid $FeSO_4$ (100 ml. of 2M- $FeSO_4$ in 0.5N-HCl/l. of ether) and distilled over NaOH pellets.

METHODS

Cerebral-cortex slices were prepared by the method of McIlwain (1951) and the top slices from each hemisphere of four young guinea pigs were used in each experiment. The slices were weighed (average slice weight 150 mg.) and

placed in Warburg vessels (two slices/vessel) containing 3 ml. of the incubation medium (Elliot's saline buffer, pH 7.4; Simon *et al.* 1967). The vessels were incubated for 1.5 hr. under an O_2 atmosphere and the CO_2 evolved was trapped in the centre well. After 1.5 hr. the reaction was stopped and the centre-well material removed and determined. The tissue and medium from the appropriate vessels were pooled and homogenized, and the amino acids and citric acid-cycle intermediates were extracted, separated and determined. The details of the methods were as described by Simon *et al.* (1967). The only change in the incubation conditions involved the substrate, [^{14}C]glutamic acid (20 μ moles/vessel, 3 μ C) being utilized as the sole added substrate in place of aspartate plus glucose.

Isolation and desalting of aspartate. The aspartate was isolated from a Dowex 50 column (2.5 cm. $\times$ 155 cm.) mounted on an automatic amino acid analyser (Beckman Instrument Co., Palo Alto, Calif., U.S.A.). The appropriate fraction containing aspartate was desalted by passing it through a Dowex 50 column, the salts were eluted with water and the amino acid was eluted with 2N- NH_3 (Simon *et al.* 1967).

Degradation of aspartate. The aspartate was converted into β -alanine by treatment with aspartate decarboxylase (General Biochemicals Inc., Chagrin Falls, Ohio, U.S.A.) by the method of Meister, Sober & Tice (1951). The CO_2 evolved from C-1 of aspartate was trapped in the centre well of a Warburg incubation vessel. The centre well contained 0.20 ml. of 0.50N-NaOH. After 1 hr. incubation the centre-well material was carefully removed, placed in a 10.0 ml. flask and made to volume with boiled distilled water. A 1 ml. sample was counted in a liquid-scintillation counter (Packard Instrument Co., La Grange, Ill., U.S.A.) and a 3 ml. sample was back-titrated to pH 8.4 with standardized HCl with a Metrohm Automatic titrator (Metrohm Ltd., Herisau, Switzerland). The resulting β -alanine was also isolated from a Dowex 50 column (2.5 cm. $\times$ 155 cm.) and

desalted on a Dowex 50 column. The β -alanine was converted into propionic acid, which was degraded stepwise by the Schmidt reaction (Finlayson, 1966).

RESULTS

Table 1 lists the relative specific radioactivities of several amino acids isolated from brain slices after incubation of the slices with radioactive glutamate. The aspartate that was isolated after incubation with [^{14}C]glutamate had a relative specific radioactivity of 0.92 whereas the aspartate isolated after incubation with [^{14}C]glutamate had a relative specific radioactivity of 0.70. The aspartate isolated after incubation with [^{14}C]glutamate had a relative specific radioactivity of 0.04. This latter finding agrees with the work of Haslam & Krebs (1963), contrary to the earlier

report by Cohen *et al.* (1962). Earlier results indicating higher relative specific radioactivities in isolated aspartate after incubation with [^{14}C]glutamate were in error owing to contamination of the aspartate isolated from the column by a related radioactive compound present in the purchased glutamate. Once discovered, this contaminant was removed for all subsequent studies (the contaminant has not yet been identified).

The relative specific radioactivities of the isolated citric acid-cycle intermediates are listed in Table 2, and in all cases are below 0.20.

The relative amounts of label found in the various carbon atoms of aspartate are listed in Table 3. When [^{14}C]glutamate was used as substrate the label was equally distributed between C-1 and C-4 of aspartate. When [^{14}C]glutamate was the added substrate the isolated aspartate had the label distributed almost evenly among all four carbon atoms; however, there is a suggestion of more label at C-2 and C-3 than at C-1 and C-4.

DISCUSSION

The conversion of the carbon skeleton of glutamate into that of aspartate has been assumed to proceed by mechanisms involving the citric acid-cycle. These conclusions arose mainly from two related findings. First, when tissue preparations were incubated with glutamate as substrate, the concentration of aspartate in the preparations rose as the glutamate content diminished (Krebs, 1935, 1950; Krebs & Bellamy, 1960; Borst, 1962). Secondly, when malonate was added to the above media, the aspartate content did not rise (Borst, 1962; Haslam & Krebs, 1963). On the basis of these findings one would expect the relative specific radioactivity [(counts/min./ $\mu\text{g. atom of C of aspartate})/(\text{counts/min./}\mu\text{g. atom of C of glutamate})]$ of aspartate that arose from [^{14}C]glutamate to equal the relative specific radioactivity [(counts/min./ $\mu\text{mole of aspartate})/(\text{counts/min./}\mu\text{mole of glutamate})]$ of aspartate that arose from [^{14}C]glutamate. This assumption would appear valid

Table 1. *Relative specific radioactivities of amino acids isolated after incubation of guinea-pig cerebral-cortex slices with [^{14}C]glutamic acid)*

Slices were incubated under O_2 for 1.5 hr. with $3.0\mu\text{C}$ of [^{14}C]glutamic acid ($20\mu\text{moles}$) and 2.8 ml. of the following saline solution: 98 mm-NaCl, 27 mm-KCl, 1.2 mm-MgSO₄, 0.4 mm-KH₂PO₄ and 17.5 mm-Na₂HPO₄, pH 7.4. Amino acids were isolated on a Dowex 50 column. Relative specific radioactivities for the experiment with [^{14}C]glutamic acid are expressed as (counts/min./ $\mu\text{g. atom of C of isolated compound})/(\text{counts/min./}\mu\text{g. atom of C of added glutamate})$; those for experiments with [^{14}C] or [^{14}C]glutamate are expressed as (counts/min./ $\mu\text{mole of isolated compound})/(\text{counts/min./}\mu\text{mole of added glutamate})$. Results are expressed as means $\pm$ s.d. of three observations.

	Relative specific radioactivity		
	[^{14}C]- Glutamate	[^{14}C]- Glutamate	[^{14}C]- Glutamate
Aspartate	0.92 $\pm$ 0.07	0.04 $\pm$ 0.04	0.70 $\pm$ 0.08
Alanine	0.52 $\pm$ 0.11	—	—
γ -Aminobutyrate	0.25 $\pm$ 0.08	—	—

Table 2. *Relative specific radioactivities of citric acid-cycle intermediates isolated after incubation of cerebral-cortex slices with [^{14}C]glutamate*

Relative specific radioactivities are expressed as (counts/min./ $\mu\text{g. atom of C of isolated compound})/(\text{counts/min./}\mu\text{g. atom of C of added glutamate})$.

Isolated intermediate	Relative specific radioactivity
Pyruvate (includes oxaloacetate)	0.19
α -Oxoglutarate	0.17
Succinate	0.16
Fumarate	0.13
Citrate	0.10

Table 3. *Labelling of aspartate obtained after incubation of slices with [^{14}C]glutamate*

Aspartate was isolated on an automatic amino acid analyser after incubation of the slices with [^{14}C]glutamate labelled in the position indicated. See the text for details. Results are the means of two observations.

Substrate	Distribution of label in aspartate (%)			
	C-1	C-2	C-3	C-4
[^{14}C]Glutamate	49.0	0	0.9	50.1
[^{14}C]Glutamate	21.9	27.8	28.2	22.1

since C-1 of glutamate is lost when glutamate is converted into aspartate by citric acid-cycle mechanisms.

The finding of Cohen *et al.* (1962) that the relative specific radioactivity of aspartate derived from [5-¹⁴C]glutamate was lower than that of aspartate derived from [U-¹⁴C]glutamate was confirmed in the present investigation. The observation that the relative specific radioactivities of the citric acid-cycle intermediates (Table 2) were lower than that of the isolated aspartate when [¹⁴C]glutamate was used as substrate for the incubation (Table 1) was also confirmed. From these findings alone one might question the assumption that glutamate is converted into aspartate by mechanisms involving the citric acid cycle. However, when [U-¹⁴C]- and [5-¹⁴C]-glutamate were used as substrates and the resulting isolated aspartate was degraded (Table 3) the label appeared in the positions that one would predict for this interconversion if the citric acid cycle were involved. These apparently divergent results may be reconciled by consideration of the theory of 'compartmentation' (Berl, Takagaki, Clarke & Waelsch, 1962*a,b*; Simon *et al.* 1967).

The carbon skeleton of glutamate would be converted into that of aspartate by citric acid-cycle mechanisms and essentially all of the isolated aspartate would be labelled. On the other hand, when the citric acid-cycle components are isolated, only those intermediates in the compartments involved in the conversion of the carbon skeleton of glutamate into that of aspartate would be labelled. Dilution of the labelled intermediates with the unlabelled intermediates will yield compounds with a lower specific radioactivity than the aspartate.

The only result that cannot be explained by this hypothesis is the difference in relative specific radioactivity of aspartate derived from [U-¹⁴C]- and [5-¹⁴C]-glutamate. It may be noted in Table 3 that there is slightly more label at C-2 and C-3 than at C-1 and C-4. Though the differences are small,

their constancy is suggestive of the explanation advanced by Haslam & Krebs (1963). These workers suggested that there may be an exchange reaction at oxaloacetate with pyruvate catalysed by a carbon dioxide-fixing enzyme. This would indeed result in the formation of aspartate that contained more label at C-2 and C-3 than at C-1 and C-4.

The results of the present study are consistent with the hypothesis that the conversion of the carbon skeleton of glutamate into that of aspartate proceeds by mechanisms involving the citric acid cycle but that this conversion is restricted to certain compartments. In considering compartmentation of amino acid and glucose metabolism it must be noted that the nature of these compartments is not yet known. In fact, the possibility cannot be dismissed that this compartmentation is an artifact and is really due to permeability problems of the free amino acids to certain cell types or subcellular particles.

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